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Quantifying Atherogenic Lipoproteins: Current and Future Challenges in the Era of Personalized Medicine and Very Low Concentrations of LDL Cholesterol. A Consensus Statement from EAS and EFLM

Langlois, Michel R ; Chapman, M John ; Cobbaert, Christa ; et al ; von Eckardstein, Arnold

Abstract: **BACKGROUND** The European Atherosclerosis Society-European Federation of Clinical Chemistry and Laboratory Medicine Consensus Panel aims to provide recommendations to optimize atherogenic lipoprotein quantification for cardiovascular risk management. **CONTENT** We critically examined LDL cholesterol, non-HDL cholesterol, apolipoprotein B (apoB), and LDL particle number assays based on key criteria for medical application of biomarkers. () **Analytical performance:** Discordant LDL cholesterol quantification occurs when LDL cholesterol is measured or calculated with different assays, especially in patients with hypertriglyceridemia >175 mg/dL (2 mmol/L) and low LDL cholesterol concentrations <70 mg/dL (1.8 mmol/L). Increased lipoprotein(a) should be excluded in patients not achieving LDL cholesterol goals with treatment. Non-HDL cholesterol includes the atherogenic risk component of remnant cholesterol and can be calculated in a standard nonfasting lipid panel without additional expense. ApoB more accurately reflects LDL particle number. () **Clinical performance:** LDL cholesterol, non-HDL cholesterol, and apoB are comparable predictors of cardiovascular events in prospective population studies and clinical trials; however, discordance analysis of the markers improves risk prediction by adding remnant cholesterol (included in non-HDL cholesterol) and LDL particle number (with apoB) risk components to LDL cholesterol testing. () **Clinical and cost-effectiveness:** There is no consistent evidence yet that non-HDL cholesterol-, apoB-, or LDL particle-targeted treatment reduces the number of cardiovascular events and healthcare-related costs than treatment targeted to LDL cholesterol. **SUMMARY** Follow-up of pre- and on-treatment (measured or calculated) LDL cholesterol concentration in a patient should ideally be performed with the same documented test method. Non-HDL cholesterol (or apoB) should be the secondary treatment target in patients with mild to moderate hypertriglyceridemia, in whom LDL cholesterol measurement or calculation is less accurate and often less predictive of cardiovascular risk. Laboratories should report non-HDL cholesterol in all standard lipid panels.

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Quantifying Atherogenic Lipoproteins: Current and Future Challenges in the Era of Personalized Medicine and Very Low Concentrations of LDL Cholesterol. A Consensus Statement from EAS and EFLM

Michel R. Langlois,^{1*} M. John Chapman,² Christa Cobbaert,³ Samia Mora,⁴ Alan T. Remaley,⁵ Emilio Ros,⁶ Gerald F. Watts,⁷ Jan Borén,⁸ Hannsjörg Baum,⁹ Eric Bruckert,¹⁰ Alberico Catapano,¹¹ Olivier S. Descamps,¹² Arnold von Eckardstein,¹³ Pia R. Kamstrup,¹⁴ Genovefa Kolovou,¹⁵ Florian Kronenberg,¹⁶ Anne Langsted,¹⁴ Kari Pulkki,¹⁷ Nader Rifai,¹⁸ Grazyna Sypniewska,¹⁹ Olov Wiklund,⁸ and Børge G. Nordestgaard,¹⁴ for the European Atherosclerosis Society (EAS) and the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) Joint Consensus Initiative

BACKGROUND: The European Atherosclerosis Society–European Federation of Clinical Chemistry and Laboratory Medicine Consensus Panel aims to provide recommendations to optimize atherogenic lipoprotein quantification for cardiovascular risk management.

CONTENT: We critically examined LDL cholesterol, non-HDL cholesterol, apolipoprotein B (apoB), and LDL particle number assays based on key criteria for medical application of biomarkers. (a) Analytical performance: Discordant LDL cholesterol quantification occurs when LDL cholesterol is measured or calculated with different assays, especially in patients with hypertriglyceridemia >175 mg/dL (2 mmol/L) and low LDL cholesterol concentrations <70 mg/dL (1.8 mmol/L). Increased lipoprotein(a) should be excluded in patients not achieving LDL cholesterol goals with treatment. Non-HDL cholesterol includes the atherogenic risk component of remnant cholesterol and can be calculated in a standard non-fasting lipid panel without additional expense. ApoB more accurately reflects LDL particle number. (b) Clinical performance: LDL cholesterol, non-HDL cholesterol, and

apoB are comparable predictors of cardiovascular events in prospective population studies and clinical trials; however, discordance analysis of the markers improves risk prediction by adding remnant cholesterol (included in non-HDL cholesterol) and LDL particle number (with apoB) risk components to LDL cholesterol testing. (c) Clinical and cost-effectiveness: There is no consistent evidence yet that non-HDL cholesterol-, apoB-, or LDL particle-targeted treatment reduces the number of cardiovascular events and healthcare-related costs than treatment targeted to LDL cholesterol.

SUMMARY: Follow-up of pre- and on-treatment (measured or calculated) LDL cholesterol concentration in a patient should ideally be performed with the same documented test method. Non-HDL cholesterol (or apoB) should be the secondary treatment target in patients with mild to moderate hypertriglyceridemia, in whom LDL cholesterol measurement or calculation is less accurate and often less predictive of cardiovascular risk. Laboratories should report non-HDL cholesterol in all standard lipid panels.

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¹ Department of Laboratory Medicine, AZ St-Jan, Brugge, and University of Ghent, Belgium; ² National Institute for Health and Medical Research (INSERM), and Endocrinology-Metabolism Service, Pitié-Salpêtrière University Hospital, Paris, France; ³ Department of Clinical Chemistry and Laboratory Medicine, Leiden University Medical Center, Leiden, the Netherlands; ⁴ Divisions of Preventive and Cardiovascular Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA; ⁵ Lipoprotein Metabolism Section, Cardiovascular-Pulmonary Branch, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD; ⁶ Lipid Clinic, Department of Endocrinology and Nutrition, Institut d'Investigacions Biomèdiques August Pi Sunyer, Hospital Clínic, Barcelona and Cíber Fisiopatología de la Obesidad y Nutrición (CIBEROBN), Instituto de Salud Carlos III (ISCIII), Spain; ⁷ Lipid Disorders Clinic, Department of Cardiology, Royal Perth Hospital, University of Western Australia, Perth, Australia; ⁸ Sahlgrenska University Hospital, Gothenburg, Sweden; ⁹ Institute for Laboratory Medicine, Blutdepot und Krankenhaushygiene, Regionale Kliniken Holding RKH GmbH, Ludwigshafen, Germany; ¹⁰ Pitié-Salpêtrière University Hospital, Paris, France; ¹¹ Depart-

ment of Pharmacological and Biomolecular Sciences, University of Milan, Italy; ¹² Hôpital de Jolimont, Haine-Saint-Paul, Belgium; ¹³ Institute for Clinical Chemistry, University Hospital Zurich, Zurich, Switzerland; ¹⁴ Herlev and Gentofte Hospital, Copenhagen University Hospital, University of Copenhagen, Copenhagen, Denmark; ¹⁵ Cardiology Department, Onassis Cardiac Surgery Center, Athens, Greece; ¹⁶ Department of Medical Genetics, Molecular and Clinical Pharmacology, Division of Genetic Epidemiology, Medical University of Innsbruck, Innsbruck, Austria; ¹⁷ Department of Clinical Chemistry, University of Turku and Turku University Hospital, Turku, Finland; ¹⁸ Boston Children's Hospital, Harvard Medical School, Boston, MA; ¹⁹ Department of Laboratory Medicine, Collegium Medicum, NC University, Bydgoszcz, Poland.

* Address correspondence to this author at: Department of Laboratory Medicine, AZ St-Jan, Ruddershove 10, B-8000 Brugge, Belgium. Fax +32-50-452619; e-mail michel.langlois@azsintjan.be.

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Table 1. Current challenges for LDLC quantification.

Cause	Problem	Recommendation ^a
Analytical		
Novel therapies: very low LDLC concentrations	Magnification of measurement and calculation errors (e.g., Friedewald)	CBR2, CBR3, CBR4
Nonfasting lipid testing	Postprandial variation of TG in LDLC calculation	CBR4, CBR5
Increasing prevalence of obesity, diabetes, and moderate or major increases in TG	Nonspecificity bias in hypertriglyceridemic (>175 mg/dL; >2 mmol/L) and dyslipidemic samples	CBR2, CBR3, CBR4, CBR9, FR1, FR2
High Lp(a)	Overestimation of LDLC	CBR10
Clinical		
Increasing prevalence of obesity and diabetes	LDLC is a less predictive marker	CBR1, CBR5, CBR6, CBR7, FR3
Residual (on-treatment) CVD risk	Residual risk unexplained by LDLC	CBR8, FR3, FR4
Personalized medicine	LDLC has low or no diagnostic and predictive performance in certain patients	CBR1, CBR8, FR4, FR5

^a CBR and future research recommendation (FR) to address the problem, listed in Table 2 (CBR) and Table 8 (FR).

Measurement of LDL cholesterol (LDLC)²⁰ is a key component in the assessment of risk of cardiovascular disease (CVD) and the management of dyslipidemia (1–4). Indeed, the causality of LDL particles in the pathophysiology of atherosclerotic CVD is indisputable (5). Furthermore, there is a direct graded relationship between LDLC concentration and the incidence of CVD observed in randomized controlled trials and metaanalyses (5, 6). All guidelines concur that lowering LDLC to concentrations below a target of 70 mg/dL (1.8 mmol/L) (or by $\geq 50\%$ if this target cannot be attained) is of critical importance in subjects at high or very high risk of CVD (1–4).

Despite the overwhelming evidence that LDLC-targeted strategies effectively reduce CVD, there is substantial between-subject variability in the response to lipid-lowering therapies and the reduction of CVD risk (7). Furthermore, accumulating evidence indicates that a focus solely on the assessment and management of LDLC

is not an optimal strategy for all patients, in part as emerging evidence has established that VLDL, their remnants, and lipoprotein(a) [Lp(a)] likewise are causally related to CVD (8–11). Major concerns equally relate to the potential for substantial errors in risk estimation given recognized LDLC measurement or calculation errors in patients with moderate or marked hypertriglyceridemia or low LDLC concentrations (Table 1). Clearly, additional biomarkers beyond LDLC are needed to identify and treat more persons at high CVD risk, especially in this era of novel therapies, such as proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibition, which efficaciously target and reduce concentrations of atherogenic lipoprotein particles to unprecedented low levels (11, 12).

For many years, the inaccuracy in measured or calculated LDLC could be tolerated because of limited clinical impact at the average to high LDLC range. However, this issue must be readdressed in the contemporary treatment era, in which much lower LDLC concentrations are seen (12) and moderate hypertriglyceridemia is potentially a greater problem because of the increasing prevalence of obesity, metabolic syndrome, and diabetes mellitus (13, 14). Furthermore, limitations of direct LDLC assays depend on the type of assay. Despite the widespread belief that direct LDLC measurements are standardized and unequivocal, data indicate that results can vary significantly between different assays from different manufacturers (15). Difficulties encountered with HDL cholesterol (HDL) assays also raise concerns about the reliability of calculated LDLC and non-HDL, as HDL is used in both calculations (15).

²⁰ Nonstandard abbreviations: LDLC, low-density lipoprotein cholesterol; CVD, cardiovascular disease; Lp(a), lipoprotein(a); PCSK9, proprotein convertase subtilisin/kexin type 9; HDL, high-density lipoprotein cholesterol; LDLP, LDL particle number; apoB, apolipoprotein B; CBR, consensus-based recommendation; dLDL, direct LDL cholesterol measurement; cLDL, calculated LDL cholesterol; TC, total cholesterol; TG, triglycerides; LPL, lipoprotein lipase; CETP, cholesteryl ester transfer protein; CHD, coronary heart disease; IDL, intermediate-density lipoprotein; Remnant-C, remnant lipoprotein cholesterol; NMR, nuclear magnetic resonance; KIV-2, kringle IV type 2; CRMLN, Cholesterol Reference Method Laboratory Network; dHDL, direct HDL cholesterol measurement; NCEP, National Cholesterol Education Program; EQA, external quality assessment; VLDL, very low-density lipoprotein cholesterol; HR, hazard ratio; RR, relative risk; MESA, Multi-Ethnic Study of Atherosclerosis; VLDLP, VLDL particle number; OR, odds ratio.

Table 2. Key CBR to improve the clinical use of atherogenic lipoprotein assays.

CBR1	Comprehensive assay(s) of atherogenic lipoproteins should assess the risk conferred by LDL particles, remnant particles, and Lp(a).
CBR2	Laboratories and clinical trial centers should report lipid profiles with declaration of the assay method/manufacturer used.
CBR3	Follow-up of lipid profiles of a patient, from baseline at diagnosis to on-treatment measurements, should be ideally performed with the same assay method (and preferably the same laboratory and instrument).
CBR4	Values near the treatment decision cutpoints should be confirmed by ≥ 2 repeated measurements by the same method and then averaged.
CBR5	Laboratories should automatically calculate and report non-HDLc on all lipid profiles.
CBR6	Non-HDLc adds Remnant-C to LDLc and can be calculated in the fasting and nonfasting state, independent of TG variability.
CBR7	ApoB assay can estimate LDLP ($\sim 95\%$ of apoB) plus Remnant-P and Lp(a) particle numbers in the fasting and nonfasting state.
CBR8	LDLc is the primary target of lipid-lowering therapy. When LDLc goal is achieved, then non-HDLc or apoB should be preferred as secondary treatment targets in patients with TG >175 mg/dL (>2 mmol/L), obesity, metabolic syndrome, or type 2 diabetes.
CBR9	When LDLc is unavailable because of an invalid Friedewald equation (TG >400 mg/dL; 4.5 mmol/L), follow-up calculation of non-HDLc should be used at higher TG concentrations rather than additional direct LDLc measurement.
CBR10	Lp(a)-corrected LDLc should be assessed at least once in patients with suspected or known high Lp(a), or if the patient shows a poor response to LDL-lowering therapy.

In addition to analytical limitations, there is clinical concern regarding the failure to prevent a large proportion of CVD events that occur despite aggressive LDLc-targeted statin therapy. Many individuals experience CVD-related events or progression of atherosclerosis despite having optimal LDLc even at concentrations <70 mg/dL (16). This residual or “hidden” risk—not identifiable by LDLc—contributes substantially to CVD-related morbidity and mortality and underscores the need for a personalized medicine approach using additional markers to better understand and manage interindividual heterogeneity (17, 18). These markers include LDL subclasses and particle concentration (LDLP), apolipoprotein B (apoB) and mass spectrometry-based proteomics, non-HDLc, remnant cholesterol and particle concentration, Lp(a), renal function and inflammation biomarkers, among others (17, 18).

The analytical validity of these markers and their incremental value beyond LDLc is strongly debated among laboratory professionals and clinicians. Other expert panels have undertaken efforts to investigate emerging biomarkers that are not related to lipid metabolism (17, 18). The current multidisciplinary consensus panel has been established by the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) and the European Atherosclerosis Society (EAS) with the aim of critically addressing the key issues of the lipoprotein and apolipoprotein markers identified above, as well as reaching a consensus on contemporary laboratory testing

for CVD risk assessment and management of lipid therapies. This article embodies the consensus-based recommendations (CBRs) of this expert panel, summarized in Table 2.

Which Atherogenic Lipoproteins Should Be Measured?

Are we using the appropriate biomarker? Standard LDLc assays measure the cholesterol content of LDL particles, expressed as milligrams per deciliter (or mmol/L) of cholesterol. LDLc concentration can be measured directly (dLDLc) with several different assays, but is still often calculated (cLDLc) from a standard lipid profile that includes measurements of total cholesterol (TC), HDLc, and triglycerides (TG). Fasting blood samples have been the standard for determining lipids because measuring them in the fasting state reduces variability of TG concentrations, thus allowing for a more accurate cLDLc estimation with the Friedewald equation. When the initial clinical decision cutpoints were developed, they were mainly derived from population studies using fasting samples. Thus, recommendations about use of fasting samples in patient care were made to ensure that results and cutpoints are comparable with those used in these studies. However, fasting is no longer routinely required for the determination of a lipid profile (19, 20). Nonfasting lipid profiles are now endorsed by several societies’

guidelines, including those in Europe, Canada, and the US (1, 3, 4).

Findings from population studies show that despite minor postprandial increases in TG and remnant cholesterol, quantitative changes in other lipids, lipoproteins, and apolipoproteins appear to be negligible in response to the habitual meal intake for most individuals (19–21). Nonfasting lipid profiles are, as we have earlier recommended (19), likely to be more relevant to the estimation of an individual's CVD risk than fasting lipids (22), including TG (23), because in real life we spend most of our time in a postprandial state (20). However, even when measured in the nonfasting state, LDLC alone does not account for all the risk conferred by atherogenic lipoproteins described hereafter. Please note that although we use the term *atherogenic lipoproteins* when we refer jointly to LDL, remnant lipoproteins, and Lp(a), this does not imply that the mechanism by which these lipoproteins cause CVD is identical.

REMNANT PARTICLES

Postprandial accumulation of TG-rich remnant particles in blood is an important factor in atherogenesis (8, 9, 24). These lipoproteins contain a higher load of cholesterol that is not considered in typical fasting TG- or LDLC-related risk estimations. TG-rich chylomicrons secreted from the intestine, as well as VLDL secreted primarily from the liver, are remodeled in the circulation primarily through the actions of lipoprotein lipase (LPL), hepatic lipase, and cholesteryl ester transfer protein (CETP). The hydrolysis of TG by LPL and acquisition of cholesteryl esters from HDL by CETP generate smaller remnant particles that are depleted of part of their TG content. Consequently, TG-rich lipoproteins also encompass cholesterol-enriched remnants—with the rare exception in individuals with familial chylomicronemia owing to complete deficiency of LPL or 1 of its key cofactors such as apoC-II and apoA-V (estimated incidence, 1/million) (13). There is persuasive experimental evidence that these remnant particles may enter the arterial intima and contribute to atherosclerosis, whereas nascent chylomicrons and very large VLDL particles are too large initially to cross the endothelial layer (8, 24). Unlike LDL particles, which need to be modified (e.g., by oxidation) to generate ligands of the macrophage scavenger receptor, TG-rich remnants can be taken up directly (without modification) by monocyte-derived macrophages, leading to the formation of foam cells, a key step in development of atherosclerotic plaques. Another mechanism by which these particles may predispose an individual to CVD involves the concept that LPL activity catalyzes the release of free fatty acids from TG-rich remnant particles, resulting in local endothelial injury and arterial inflammation (24). Mendelian randomization studies suggest that life-long high plasma concentrations

of TG-rich lipoproteins or their remnants are causally associated with increased risk of coronary heart disease (CHD) (25–28) and all-cause mortality (29).

Accurate isolation and quantification of remnants has previously been problematic, as remnants are difficult to differentiate from their larger and more TG-rich precursors; furthermore, their plasma concentration is typically much lower compared with other lipoproteins. There are several early assays that claim to specifically measure cholesterol in remnants, but they show poor agreement (30). Some have been validated in cohort studies and have revealed significant associations of remnant cholesterol with cardiovascular events (31, 32).

Larger data sets have been obtained by the calculation of “remnant cholesterol” ($\text{Remnant-C} = \text{TC} - \text{HDL-C} - \text{LDLC}$) because Remnant-C corresponds to all cholesterol not found in LDL and HDL, i.e., in all TG-rich lipoproteins (Fig. 1). In the fasting state, this constitutes cholesterol in VLDL and intermediate-density lipoproteins (IDLs), whereas in the nonfasting state, a relatively small amount of cholesterol can also be found in chylomicron remnants. Because both newly secreted chylomicrons and VLDL are acted on rapidly by LPL, any circulating chylomicrons and VLDL have undergone some partial lipolysis and hence can be considered remnants (24). If Friedewald-cLDLC is used in the calculation, then Remnant-C simply equals $\text{TG}/2.2$ (in mmol/L) or $\text{TG}/5$ (in mg/dL) and does not provide any clinical information beyond TG concentrations; however, this is not the case if dLDLC is used in the calculation. Importantly, a homogeneous direct assay to measure Remnant-C in all TG-rich lipoproteins combined has become available to be used with standard hospital autoanalyzers, and direct Remnant-C is highly correlated with calculated Remnant-C, although not identical (33).

Remnant-C also contributes to non-HDL-C, which is calculated as the difference between TC and HDLC (19). This term is independent of the Friedewald term and, therefore, not confounded as much with TG concentrations as calculated Remnant-C; thus, it represents an additional clinically valuable marker (19). Remnant-C, measured or calculated, differs from non-HDL-C in that non-HDL-C contains Remnant-C plus LDL-C (Fig. 1). Some individuals with high Remnant-C have low LDL-C, and if interpreting non-HDL-C instead of Remnant-C, then high Remnant-C will be masked in these individuals.

LDL PARTICLES

All LDL particles are highly atherogenic, but their concentration is not always reflected by LDL-C measurements, particularly in hypertriglyceridemic patients with diabetes or related conditions such as visceral obesity and insulin resistance, which are critical components of the metabolic syndrome (13). LDL-C concentration does not automatically equal LDLP because the cholesterol/TG

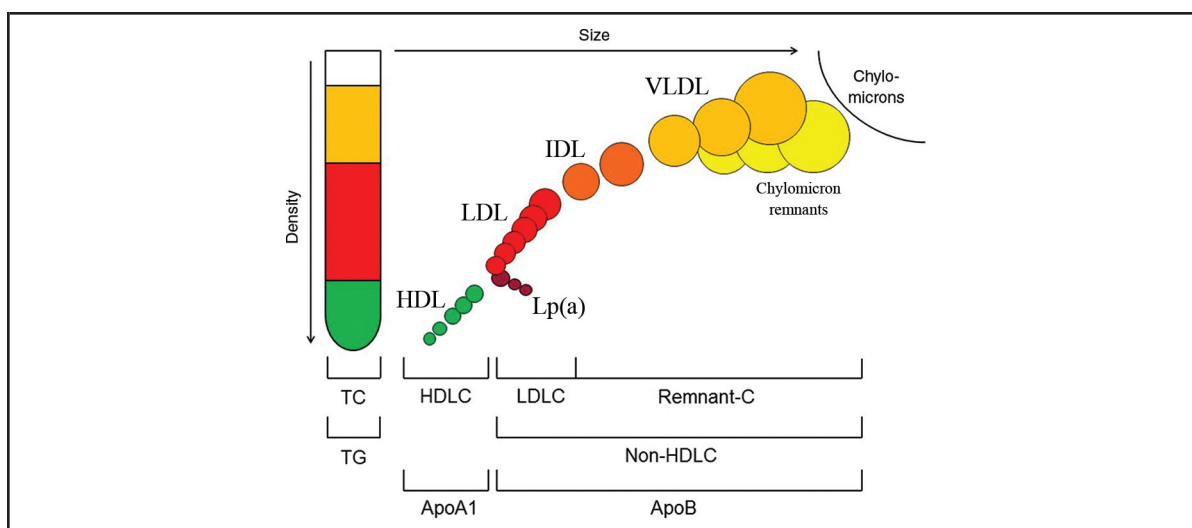


Fig. 1. Lipoproteins separated according to density and size and their representative laboratory markers measured in a blood sample.

Standard lipid profiles consist of measurements of TC, TG, HDLC, and LDLC; however, a standard lipid profile could also report calculated Remnant-C and calculated non-HDL-C. Remnant-C, calculated as $TC - HDLC - LDLC$, is all cholesterol not found in LDL and HDL, i.e., in all TG-rich lipoproteins: VLDL, IDL, and, in the nonfasting state, chylomicron remnants. Non-HDL-C, calculated as $TC - HDLC$, represents a comprehensive measure of all cholesterol found in atherogenic lipoproteins: LDLC, Remnant-C, and Lp(a) cholesterol. ApoB and apoA1 can be used as alternatives to non-HDL-C and HDLC. The cholesterol content of Lp(a), corresponding to approximately 30% of Lp(a) total mass, is included in TC, non-HDL-C, and LDLC measurements and its apoB content in the apoB measurement.

ratio in the particles can vary widely between individuals, reflecting differences in LDL subfraction profile (34). Small LDL particles contain less (LDL) cholesterol than large ones. Although LDLC is typically not increased in patients with type 2 diabetes, such patients tend to have smaller average LDL particle size and will have concomitantly more LDL particles than a patient with the same LDLC concentration who has larger average LDL size (34). These small LDL particles, typically predominant in those with mild to moderate hypertriglyceridemia, are the products of intravascular remodeling of larger TG-rich VLDL particles by 2 processes: first, the progressive hydrolysis of TG-rich VLDL by LPL; second, the increased exchange and transfer of TG and cholesteryl ester mediated by CETP to produce TG-enriched LDL particles (13). TGs are then hydrolyzed by hepatic lipase, resulting in smaller and denser LDL particles with less cholesteryl ester per particle. These compact, lipid-depleted LDL particles are less efficiently cleared via hepatic LDL receptors (35), leading to higher LDLP in patients with increased TG than would be predicted based on LDLC measurement. Concomitantly, TG-enriched HDL resulting from the action of CETP is also modified by hepatic lipase, producing smaller HDL and contributing to lower concentrations of HDLC, as typically manifested in the atherogenic dyslipidemic triad

involving hypertriglyceridemia, increased small dense LDLP, and low HDLC (35).

Measurement of apoB, the major protein component of LDL, can also be used to assess the number of LDL particles (36); however, an apoB measurement also includes Lp(a), IDL, VLDL, and chylomicron remnants (Fig. 1). ApoB measurement is not, however, usually part of the standard lipid profile. Monogenic disorders that impair the removal of LDL particles from the circulation, such as familial hypercholesterolemia, can be easily recognized from the standard lipid profile without apoB. In contrast, polygenic hypertriglyceridemia or combined hyperlipidemia can be more adequately characterized based on TG and apoB (37). Although not widely available, LDLP measured by nuclear magnetic resonance (NMR) spectroscopy provides an alternative measure of the number of LDL particles and has been shown to be at least equivalent to apoB and non-HDL-C in predicting cardiovascular risk (38).

Lp(a) PARTICLES

Lp(a) is an LDL-like particle with 1 molecule of apoB to which an additional apolipoprotein, apo(a), is covalently attached. This apolipoprotein shows considerable size polymorphism originating from a variable number of kringle IV type 2 (KIV-2) repeats of apo(a), as encoded

Table 3. CBRs for the clinical indication for atherogenic lipid and lipoprotein quantification.

	CVD risk estimation	Dyslipidemia characterization	Treatment choice	Treatment target	Desirable value
TC	Yes ^a	Optional ^b	Optional ^b	Optional ^b	<190 mg/dL (5.0 mmol/L)
LDLC	Yes	Yes	Yes	Yes	Low to moderate risk <115 mg/dL (3.0 mmol/L)
					High risk <100 mg/dL (2.5 mmol/L)
					Very high risk <70 mg/dL (1.8 mmol/L)
TG	Yes	Yes	Yes	No	Fasting <150 mg/dL (1.7 mmol/L)
					Nonfasting <175 mg/dL (2.0 mmol/L)
Non-HDLC	Yes	No	No	Yes ^c	Moderate risk <145 mg/dL (3.8 mmol/L)
					High risk <130 mg/dL (3.3 mmol/L)
					Very high risk <100 mg/dL (2.5 mmol/L)
ApoB ^d	Optional ^c	Yes ^c	No	Optional ^c	High risk <100 mg/dL (1.0 g/L)
					Very high risk <80 mg/dL (0.8 g/L)

^a In combination with HDLC.
^b To be considered when LDLC is not available.
^c In patients with mild to moderate hypertriglyceridemia (2–10 mmol/L; 175–880 mg/dL).
^d Or LDLP if available.
 To convert mmol/L to mg/dL, multiply by 38.6 for cholesterol and 88.5 for TG.

by tandem repeats of a genomic sequence in the *LPA*²¹ gene (10, 39). This size polymorphism is the most important determinant of the hepatic production rate of Lp(a), and results in marked interindividual variation of plasma Lp(a) concentrations by >1000-fold. Individuals expressing a low number of KIV-2 repeats [small apo(a) phenotypes] show, on average, markedly higher Lp(a) concentrations than those with a high number of KIV-2 repeats [large apo(a) phenotypes], who, on average, have low Lp(a) concentrations. An increased Lp(a) concentration is a strong genetic risk factor for CVD and calcific aortic valve stenosis independent of LDLC in the general population (10, 39, 40). One of the major differences between these 2 particles is that LDLs are effectively lowered by statins, whereas Lp(a) is typically resistant to this treatment. Although PCSK9 inhibitors and other novel agents reduce both LDLC and Lp(a) (12), it is unknown whether Lp(a) lowering per se contributes to the clinical benefit of these novel agents.

Lp(a) should be measured in all patients screened for high risk of CVD or aortic stenosis, in cases of premature CVD, and in those with a positive family history of CVD or high Lp(a) (10). It is often a likely reason of otherwise unexplained CVD cases. However, Lp(a) measurement should not be included in repeated lipid profile measurements within the same patient [as Lp(a) concentrations exhibit little variation over a lifetime], unless treatment is known to influence Lp(a) concentrations. Importantly,

the cholesterol content of Lp(a) is included in calculated and measured LDLC and, consequently, also TC and non-HDLC values (19).

Consensus-based recommendation. Comprehensive testing of atherogenic lipoproteins should use a biomarker, or a panel of multiple markers, that can be measured in either the fasting or nonfasting state and assesses the risk associated not only with LDL particles but also remnant particles and Lp(a) (CBR1). The use of atherogenic lipoprotein testing in different clinical settings such as CVD risk estimation, dyslipidemia diagnosis, risk management, and treatment has been emphasized in other guidelines (1) (Table 3).

Are LDLC Measurements or Calculations Reliable?

OPERATIONAL DEFINITION OF LDL

Most manufacturers of lipid assay kits certify and standardize their assays by comparison with a Cholesterol Reference Method Laboratory Network (CRMLN) laboratory. The CRMLN standardization process ensures that the calibrators and reagents sold by manufacturers produce test results that are traceable to the CDC reference methods, i.e., β -quantification for LDLC and ultracentrifugation/heparin-Mn²⁺ precipitation for HDLC (41).

β -Quantification requires ultracentrifugation of serum or plasma at a density of 1.006 g/mL to separate the supernatant, which contains VLDL and chylomicrons, from the infranatant, which contains LDL, HDL, and

²¹ Human Gene: *LPA*, lipoprotein(a).

Lp(a). Cholesterol is measured in the infranatant to provide the sum of LDL, HDL, and Lp(a) cholesterol, and then LDL particles, including Lp(a), are precipitated from the infranatant, and HDLC is measured in the remaining supernatant. LDLC is then calculated as infranatant cholesterol minus supernatant (HDL-) cholesterol, both measured with the Abell-Kendall cholesterol reference method (41). However, it is not widely recognized that this LDLC also contains the cholesterol from Lp(a), which can be substantial in the case of high Lp(a) concentrations >50 mg/dL (10).

An important prerequisite for reference standardization of the LDLC and HDLC assays is an unambiguous definition of the lipoproteins intended to be measured. With β -quantification, the lipoprotein fraction in the density range of 1.006 to 1.063 g/mL is defined as LDL, and the fraction in the density range of 1.063 to 1.21 g/mL is defined as HDL (41). Yet, these operational definitions allow variable degrees of cross-reactivity of cholesterol from IDL with a density of 1.006 to 1.019 g/mL and Lp(a) with a density of 1.04 to 1.13 g/mL in the LDLC fraction using β -quantification. Direct assays that attempt to specifically measure cholesterol in LDL may, therefore, show discordant results compared with the reference method (15).

DIRECT LDLC AND HDLC ASSAYS

In the previous century, the earliest measurements of lipoproteins involved ultracentrifugation and (most popular) precipitation techniques for isolation of LDL and HDL (42). In the late 1990s, “homogeneous” or “direct” LDLC and direct HDLC (dHDLC) methods were introduced in clinical laboratories and have since largely replaced the older assays, particularly for HDLC (15, 42). The homogeneous methods are commercially available as ready-to-use reagents, enabling fully automated dLDLC and dHDLC measurements from the primary blood sample tube, without any need for ultracentrifugation or precipitation to separate HDL and LDL particles. There are various dLDLC and dHDLC assays available based on different principles from different manufacturers to selectively isolate and measure cholesterol in these lipoproteins. Despite the ease of use and cost savings, mostly because of automation, substantial nonselectivity errors have been reported for many of the direct assays (15, 42).

According to National Cholesterol Education Program (NCEP) criteria, the total error of LDLC and HDLC measurements should be within 12% and 13%, respectively, of the true value (41, 43). The *total error* term combines 2 analytical components: imprecision and bias. Imprecision refers to the reproducibility of a method, the “random error” often reported as a CV. Bias, or inaccuracy, refers to a systematic difference in results between a method and the “true” or reference value: It should be $\leq 4\%$ for LDLC and $\leq 5\%$ for HDLC to allow

the methods to meet the total error goals (41, 43). The discordance between measurements and “true” values, assigned by the reference method, is caused by both bias (which in theory can be eliminated) and imprecision (which can only be minimized but not avoided) in the tests. The direct methods are well standardized using normal patient samples and demonstrate improved precision because of the elimination of sample pretreatment steps, but their bias is a major point of concern in case of aberrant sample matrices, e.g., owing to dyslipidemia. Unsolvable errors—regardless of the method used (ultracentrifugation, precipitation, or homogeneous assays)—relate to the ambiguity in the operational definition of “LDL” and “HDL” fractions and the heterogeneity of LDL and HDL particles. Both LDL and HDL fractions comprise different subclasses of particles that vary in size, density, shape, and lipid and apolipoprotein composition, without any definitive chemical structure, making development of specific assays difficult (15, 42). Direct assays based on different principles may select different subclasses of LDL or HDL that may or may not be equally quantified, depending on the assay procedure and reagents. The reaction specificities of dLDLC assays vary regarding reactivity to small dense LDL subfractions and nonspecific reactivity to VLDL subfractions (15). Consequently, nonspecificity bias is caused by the inaccuracy of the tests in selectively quantifying what is intended to be measured; this bias is inevitable and varies per sample (15, 42, 44).

Most discrepancies—with marked deviations from the CDC reference methods—are observed in samples from patients with hypertriglyceridemia >2 mmol/L (>175 mg/dL), mixed dyslipidemia, or other conditions involving altered lipoprotein composition and remodeling, such as diabetes and chronic kidney disease (44–46). In a comprehensive study of direct methods using fresh samples from individuals with and without CVD and/or various dyslipidemias, Miller et al. showed that only 5 of 8 dLDLC methods and only 6 of 8 dHDLC methods met the NCEP total error goal with samples from nondyslipidemic individuals, and all methods failed to meet NCEP performance criteria with those from dyslipidemic individuals (44). The total error of dHDLC and dLDLC measurements ranged approximately $\pm 13\%$ in normolipidemic samples but from -20% to $+36\%$ for dHDLC and -26% to $+32\%$ for dLDLC in dyslipidemic samples (44). Most discordant results were observed at lower concentration ranges of HDLC (<40 mg/dL) and LDLC (<70 mg/dL) (44), which are now more clinically relevant given the highly efficacious LDL-lowering therapies presently available.

Test results differ substantially between the various direct methods from different manufacturers, particularly in hypertriglyceridemic (>2 mmol/L; >175 mg/dL) samples. For all dHDLC and dLDLC methods, a

high proportion ($\geq 10\%$; for some methods, $30\%–45\%$) of test results fall outside the NCEP total error goals in dyslipidemic samples (44). We cannot recommend a specific manufacturer method, as no consistent pattern has been documented for the frequency of analytical errors with each method in normolipidemic or dyslipidemic samples. This is also evident from large-scale accuracy-based quality surveys organized across different laboratories (47, 48). In a survey of 190 US laboratories, all dLDLC methods failed the bias criterion of $\leq 4\%$ on a fresh frozen pooled serum with a TG concentration of 2.2 mmol/L (193 mg/dL) (47). An external quality assessment (EQA) study of 200 clinical laboratories in the Netherlands, representing common dLDLC and dHDL methods used in most countries, revealed unacceptable bias (up to 20%) with most manufacturers' methods in fresh and frozen serum pools, with TG concentrations of 7 mmol/L (620 mg/dL) (48). Reported dLDLC data showed values for mean bias of +16% with Abbott Diagnostics, +14% with Beckman Coulter, and –7% with Roche Diagnostics methods as compared with the CRMLN reference laboratory measurement (48). Mean dHDL biases were also method-dependent [–3% (Abbott), –7% (Beckman Coulter), –19% (Roche), and –22% (Siemens)] and contributed to between-laboratory variability of cLDLC and non-HDL calculations (48). These errors resulted in misclassifications with respect to CVD risk assessment, depending on the laboratory where LDL or HDL was measured (48).

The biases noted in dLDLC and dHDL assays when analyzing dyslipidemic samples with atypical lipoproteins suggest that nonspecific cross-reaction takes place, with high variability between the different chemical procedures used to isolate the lipoproteins intended to be measured. This shortcoming is not revealed in current CDC certification programs.

cLDLC

In most laboratories, LDLC is calculated by the Friedewald formula, cLDLC = TC – HDLC – VLDL cholesterol (VLDLC), wherein VLDLC is estimated as TG/2.2 in mmol/L or TG/5 in mg/dL (49).

Like dLDLC measurements, cLDLC calculation is not without flaws. The Friedewald formula also includes cholesterol in IDL and Lp(a) and assumes a constant VLDL TG/cholesterol ratio, lack of chylomicrons, and lack of excessive remnant lipoproteins. Nonfasting samples do not necessarily meet these assumptions, as chylomicrons may be present and are more TG-rich than VLDL particles (42). Because the TG/cholesterol ratio in TG-rich VLDL and chylomicrons progressively increases as hypertriglyceridemia and (postprandial) chylomicronemia become more severe, the equation overestimates VLDLC and, therefore, underestimates LDLC at high TG concentrations (42). In a study of type 2 diabetic

Table 4. Analytical and biological sources of error in variables included in the Friedewald-cLDLC equation.

TC
(Pre-)analytical and intraindividual (biological) variations of TC
Lp(a) cholesterol in patients with increased Lp(a) is not subtracted from TC
HDL
(Pre-)analytical and intraindividual (biological) variations of HDL
Inaccurate dHDL measurement (nonspecificity bias) in dyslipidemic samples
VLDL
TG/cholesterol ratio increases with increasing hypertriglyceridemia
Invalid use of fixed TG/cholesterol ratio in type III dyslipoproteinemia and chylomicronemia
Intraindividual (biological) variations of TG
Postprandial variations of TG, when using nonfasting samples
Increased free glycerol concentration in nonglycerol blanked TG assays ^a
^a The free or endogenous glycerol concentration in a sample can usually be ignored (1 mg/dL, equivalent to approximately 10 mg/dL (0.11 mmol/L) of TG). Increased baseline glycerol concentrations are found in patients with diabetes and chronic kidney disease, and will add to underestimation of cLDL using nonglycerol blanked TG assay.

patients, postprandial decrease in LDLC was –12% with nonfasting cLDLC vs –5% on beta-quantification at 5 h postprandially (50). Hence, cLDLC exaggerates the (usually minor) postprandial decrease in LDLC, which is clinically insignificant in most individuals (19). The equation is increasingly inaccurate at TG concentrations from 200 to 400 mg/dL (2.3–4.5 mmol/L) and is regarded as invalid when TGs are >400 mg/dL (4.5 mmol/L) or in rare cases of type III dyslipoproteinemia in which cholesterol-rich β -VLDL remnants are present and VLDLC will be underestimated (therefore, LDLC will be overestimated) (13, 42). Several alternative cLDLC equations and adjustable factors for the TG/VLDLC ratio have been proposed (51–54), but it remains to be determined as to whether improvements over the Friedewald equation improve risk prediction or are substantive enough to justify their implementation in clinical practice (55). The fact that cLDLC depends on 3 laboratory measures, i.e., TG, TC, and dHDL, means that 3 CVs are involved, which increases the potential for measurement error (Table 4).

Both imprecision and bias of cLDLC increase at lower LDLC concentrations, an aspect that is more relevant because highly effective LDL-lowering therapies, including combination therapies (e.g., statins with ezetimibe or statins

with PCSK9 inhibitors), are now available (11, 12). The original Friedewald equation in 1972 was not designed to be used in patients receiving such treatments. In a correlation analysis of pooled data from 14 trials of alirocumab-treated patients in the ODYSSEY program, only minor differences were observed between LDLC values derived by Friedewald calculation and β -quantification (56). In patients with LDLC in the range of 15 to <25 mg/dL (0.4 to <0.6 mmol/L) as measured by β -quantification, there was a median difference of 3.5 mg/dL (0.1 mmol/L) compared with cLDLC; in those with measured LDLC <15 mg/dL (0.4 mmol/L), there was a median 3-mg/dL (0.1-mmol/L) difference (56). These small differences are likely to have little clinical impact. Another report showed that underestimation of LDLC by the Friedewald equation compared with ultracentrifugation (β -quantification), especially an LDLC <70 mg/dL (1.8 mmol/L) with a median difference of -4 mg/dL (0.1 mmol/L), resulted in treatment group misclassification of 29% of patients with respect to the guideline-recommended cutpoint of 70 mg/dL (1.8 mmol/L) (55). These studies reported pooled data from predominantly (>75%) normotriglyceridemic study populations (55, 56). In a subanalysis of 33 106 hypertriglyceridemic patients with TGs of 200 to 399 mg/dL (2.3–4.5 mmol/L) from a large study sample (n = 191 333) with cLDLC ranging from 2 to <70 mg/dL (0.05 to <1.8 mmol/L), median cLDLC bias was -18 mg/dL (-0.5 mmol/L) (5th to 95th percentile, -7 to -36 mg/dL; -0.2 to -0.9 mmol/L) compared with LDLC measured by vertical spin density gradient ultracentrifugation (57). Median biases were larger at the lowest concentration ranges: -26 mg/dL (-0.7 mmol/L) at cLDLC of 15 to <25 mg/dL (0.4 to <0.6 mmol/L), and -33 mg/dL (-0.9 mmol/L) at cLDLC of <15 mg/dL (0.4 mmol/L) (58). The same investigators found that cLDLC was frequently classified as <70 mg/dL (1.8 mmol/L) despite true LDLC concentrations \geq 70 mg/dL; indeed, 39% of patients were misclassified when TG concentrations were 150 to 199 mg/dL (1.7–2.3 mmol/L), and 59% were done so when concentrations ranged from 200 to 399 mg/dL (2.3–4.5 mmol/L) (57). These patients may be excluded from treatment because of underestimated LDLC (57, 58). It should be noted that the target (i.e., 70 mg/dL; 1.8 mmol/L) is based on population studies using cLDLC and not ultracentrifugation; thus, it could be argued that ultracentrifugation (β -quantification) is the method that misclassifies risk.

These observations reflect the inaccuracy of the Friedewald equation over the full range of LDLC values seen with novel therapies. In persons with very low LDLC and concurrently high TG, VLDLC estimation (TG/5 in mg/dL or TG/2.2 in mmol/L) constitutes a relatively larger portion of the equation. In this situation, the error of overestimated VLDLC with increasing TG-rich

VLDL has a significant impact on the total error of estimated cLDLC.

EFFECT OF Lp(a) CHOLESTEROL ON LDLC

Friedewald-estimated cLDLC and most dLDLC methods include the cholesterol that is present in Lp(a) particles (15, 59). Considering that an Lp(a) particle is composed of about 30% to 45% cholesterol by weight, a substantial overestimation of TC, non-HDLc, and LDLC concentration occurs in individuals with high and very high Lp(a) concentrations (59); for example, if a person has an Lp(a) concentration of 100 mg/dL, cLDLC and dLDLC will be overestimated by 30 to 45 mg/dL (0.8–1.2 mmol/L). These circumstances might explain some cases of nonresponse or low response to statin treatment. Statins are known to have a pronounced effect on LDLC but do not lower Lp(a) concentrations (60).

If a patient receives a statin with the aim to reduce LDLC to a target of <70 mg/dL, and if that patient already has a cLDLC value of 100 mg/dL and an Lp(a) concentration of 100 mg/dL, the Lp(a)-corrected LDLC is only approximately 55 to 70 mg/dL [i.e., 100 mg/dL cLDLC minus 30%–45% of measured Lp(a)]. In the new era of potent LDLC-lowering therapies, the achieved true LDLC concentrations [after correction for Lp(a) cholesterol] can be as low as 10 mg/dL (0.3 mmol/L) (61). This scenario is particularly likely in African-Americans, who often have 2- to 3-fold higher Lp(a) concentrations than whites, or in patients with nephrotic syndrome or in those undergoing peritoneal dialysis in whom Lp(a) concentrations can reach up to >300 mg/dL, which corresponds to an Lp(a)-corrected LDLC that is 135 mg/dL (3.5 mmol/L) lower than the uncorrected LDLC value (62). For example, patients with nephrotic syndrome had, on average, 27-mg/dL higher LDLC concentrations if not corrected for cholesterol derived from Lp(a) (compared with only 9 mg/dL in controls) (62).

Lp(a)-corrected LDLC can be estimated with the Dahlen modification of the Friedewald formula, which assumes that 30% of Lp(a) weight consists of cholesterol: $cLDLC = TC - HDLC - TG/5 - [Lp(a) \times 0.30]$ in mg/dL (63). We recommend that Lp(a)-corrected LDLC be applied at least once in patients with suspected high Lp(a), or in a patient who does not respond sufficiently to statin therapy, to identify or exclude potential interference by high or very high Lp(a) in making treatment decisions (CBR10). If a high Lp(a) concentration is the cause for an apparently disappointing LDLC-lowering response with a statin, then it might not be useful to increase the dosage of statin under such conditions (60).

DISCORDANT cLDLC VS dLDLC

Measured and calculated LDLC correlate well using both fasting and nonfasting lipid profiles in general population statistics (19), but often cLDLC may not agree with dLDLC in an individual subject (15). Friedewald-cLDLC with a constant TG/cholesterol ratio cannot adjust for the postprandial increase in TG (42). β -Quantification removes TG-rich lipoproteins by ultracentrifugation before measurement, and most dLDLC assays attempt to selectively measure cholesterol in LDL particles by either blocking or solubilizing non-LDL particles (15). Thus, technically, β -quantification and dLDLC are less sensitive to TG-rich lipoproteins and should not be influenced by a nonfasting state, but the direct assays have varying specificity limitations when abnormal lipoproteins and increased TG are present (44–46).

Among 1508 men (including 173 CHD events during follow-up) and 1680 women (including 74 incident CHD events), the Framingham Study found good agreement of cLDLC with dLDLC (Kyowa Medex assay) (64). Discrepancies of >10% were assessed in 7.7% of LDLC determinations, but at higher TG concentrations and in patients with diabetes, CHD, or on cholesterol-lowering medications, there was a greater bias between the 2 methods (64). This study, as in the Women's Health Study below, did not compare either the cLDLC or the dLDLC method with a gold standard such as ultracentrifugation. The Women's Health Study ($n = 27\,331$) also observed good correlations between cLDLC and dLDLC (Roche Diagnostics assay) in fasting and nonfasting samples (65). However, the average dLDLC concentration was 5 to 10 mg/dL (0.1–0.3 mmol/L) lower than cLDLC. Associations of fasting cLDLC and dLDLC concentrations with CHD events showed similar hazard ratios (HRs) of 1.22 (95% CI, 1.14–1.30) and 1.23 (95% CI, 1.15–1.32) per 1-SD increase (35 and 34 mg/dL; 0.9 and 0.9 mmol/L), respectively. However, the lower dLDLC resulted in classification of about 20% of individuals with discrepant risk as compared with cLDLC (65).

Even in normotriglyceridemic samples, dLDLC methods do not offer advantage over cLDLC in classifying patients into NCEP risk categories when compared with the reference method (66). In a study of 145 fasting individuals with TG <200 mg/dL (2.3 mmol/L), 7 of 8 commercially available dLDLC methods failed to show improved CVD risk score classification over the corresponding cLDLC estimated using the dHDL method from each manufacturer in the calculation (66). The overall misclassification rate for the CVD risk score ranged from 5% to 17% for cLDLC methods and from 8% to 26% for dLDLC methods when compared with β -quantification, and most normotriglyceridemic individuals were classified into a lower risk category by the dLDLC methods (3%–26%) (66).

These observations suggest no substantial advantage for using dLDLC compared with cLDLC in normotriglyceridemic and hypertriglyceridemic blood samples up to TG concentrations of 400 mg/dL (4.5 mmol/L). dLDLC measurement is clearly more expensive than the “free of charge” cLDLC. Furthermore, most clinical trials demonstrating the evidence base for clinical benefit of LDLC lowering with statin therapy have used the Friedewald equation.

LDLC TEST ERRORS: ARE THEY CLINICALLY RELEVANT?

Clinical or epidemiological studies can validate whether analytical errors in LDLC testing are relevant in clinical decision-making and whether they influence outcomes. The ranges of uncertainty across different LDLC methods are not negligible (Table 5). Difficulties in treatment options may arise when LDLC test results are close to guideline-driven critical values that determine the decision of therapeutic intervention (Fig. 2). Misclassification—and, thus, inappropriate treatment—may occur if a true LDLC concentration is above the optimal target value but the reported LDLC value is in a desirable range, or if a true LDLC concentration is in a desirable range but the reported LDLC value is above target, e.g., 70 mg/dL (1.8 mmol/L), in a patient with a very high-risk score (1). The former underdiagnosis bears the risk of insufficient treatment and adverse clinical outcome; the latter overdiagnosis will increase costs by leading to unnecessary prescriptions of statins in general and specifically combination treatments.

Depending on the methods used, different treatment decisions may be taken, or confusion may arise if the patient's samples for monitoring are sent to different laboratories using different methods or when a laboratory changes the method. Not uncommonly, changes in a patient's LDLC test result over time are within the range of uncertainty of laboratory method variation and may not be because of therapeutic intervention. Between-method and between-laboratory differences in on-treatment LDLC may even mimic (or mask) a nutritional or pharmaceutical effect, e.g. ezetimibe, when values differ >15% over time when measured by different methods during follow-up (67). Based on the Cholesterol Treatment Trialists' metaanalysis (6), measurement biases of up to -0.5 mmol/L (-20 mg/dL) can be falsely interpreted as a 10% CVD risk reduction (Fig. 3). For example, in the REVEAL study, the mean LDLC reduction in the anacetrapib-treated group compared with the placebo group was -26 mg/dL (-0.68 mmol/L; -41%) as measured with a direct assay (Beckman Coulter) but only -11 mg/dL (-0.28 mmol/L; -17%) as measured on β -quantification in the same samples (68). These issues are, however, less relevant for the monitoring of the patient by the same laboratory and method over time. In this situation, the bias remains constant over time and

Table 5. Examples of interlaboratory uncertainty when plasma lipid parameters are determined by different methods.

Assay	Assumed total error	Defined concentration in model patient	Range of uncertainty
TC	9% ^a	200 mg/dL (5.2 mmol/L)	182–218 mg/dL (4.7–5.7 mmol/L)
TG	15% ^a	250 mg/dL (2.8 mmol/L)	212–288 mg/dL (2.4–3.3 mmol/L)
HDLC	–20% to +36% ^b	40 mg/dL (1.0 mmol/L)	32–54 mg/dL (0.8–1.4 mmol/L)
Non-HDLC	Derived from TC and dHDLC	160 mg/dL (4.1 mmol/L)	128–186 mg/dL (3.3–4.8 mmol/L)
Measured LDLC	–26% to +32% ^b	110 mg/dL (2.8 mmol/L)	82–145 mg/dL (2.1–3.8 mmol/L)
Estimated LDLC (Friedewald)	Derived from TC, TG, and dHDLC	110 mg/dL (2.8 mmol/L)	70–144 mg/dL (1.8–3.7 mmol/L)
ApoB	12% ^c	110 mg/dL (1.1 g/L)	97–123 mg/dL (0.9–1.2 g/L)

^a Based on NCEP analytical performance criteria (41).
^b Total error ranges observed by Miller et al. (44) across different dLDLC and dHDLC methods in dyslipidemic samples. The total error combines systematic bias and random imprecision. The tables are not relevant for the monitoring of a patient by the same laboratory/method over time. In this situation, the bias remains constant and only the (inevitable) imprecision is relevant. It will be considerably lower than the total error, at least for dHDLC and dLDLC (<10%), but not for TC, TG, and the derived measures cLDLC or non-HDLC.
^c Based on AACC Lipoprotein and Vascular Diseases Division–Working Group on Best Practices assessment (38).

only the imprecision (random error) is relevant, which may not be important given that clinicians are not aiming to achieve the LDLC targets exactly but often concentrations below it. In the present era, the extent of LDLC reduction is more important than achieving specific targets, and recent guidelines suggest that achievement of a >50% reduction in high- and very high-risk patients is paramount, irrespective of baseline LDLC concentration (2).

The risk that errors in LDLC measurement affect the clinical decision is further attenuated by the NCEP recommendation that decisions to initiate a treatment, or adjusting or shifting to another treatment, should not be taken on 1 LDLC measurement but rather after multiple repeated measurements (at least 2 times) to allow for intraindividual (biological) variation (43).

Consensus-based recommendation. Analytical performances of dLDLC and cLDLC are acceptable in blood samples with normal TG. That said, variable nonspecificity errors may confound measurements in samples with hypertriglyceridemia >175 mg/dL (2 mmol/L), which is seen in approximately 25% of individuals in the general population (24), or in samples with low LDLC <70 mg/dL (1.8 mmol/L); however, these errors may still be less than the between-method, between-laboratory, biological, and, thus, total error. We recommend that laboratories and clinical trial centers report lipid levels together with the test method used, and in a similar manner to recommendations made to report other laboratory tests used for monitoring, e.g., tumor markers, to make clinicians aware of changes in methods as a potential cause of implausible laboratory test results (CBR2). Follow-up of lipid profiles in a patient, from baseline to on-treatment measurements, should ideally be performed with the same method (and preferably in the same laboratory and

instrument) to minimize CVD-risk misclassifications (CBR3). Values near the therapeutic decision cutpoints should ideally be confirmed by repeated measurement(s) (≥2) by the same method and then averaged (CBR4).

Are Alternative Atherogenic Lipoprotein Measures Reliable?

CALCULATED NON-HDLC

Calculated by simply subtracting HDLC from TC, non-HDLC represents the cholesterol in all atherogenic particles, i.e., LDL, VLDL, IDL, chylomicron remnants, and Lp(a). In contrast to LDLC, non-HDLC considers the atherogenic potential of remnant lipoproteins (19). Therefore, non-HDLC provides a more comprehensive risk estimation than does LDLC in patients with hypertriglyceridemia by adding VLDLC (= Remnant-C) to LDLC (13).

Like LDLC, non-HDLC is managed with existing lipid-lowering agents, and there is a direct consistent relationship between the magnitude of non-HDLC lowering and CHD-risk reduction (69). In a metaanalysis of 14 statin (n = 100 827), 7 fibrate (n = 21 647), and 6 niacin (n = 4445) trials, a 1% decrease in non-HDLC was associated with a 1% decrease of relative risk (RR) for CHD (69). The estimated 1:1 relationship translated into an RR of 0.78 (95% CI, 0.64–0.94) for a 25% decrease in non-HDLC (69). In another metaanalysis of 49 statin and nonstatin trials (n = 312 175), the RR of CVD per 1-mmol/L (39 mg/dL) reduction in non-HDLC was 0.80 (0.77–0.82), which was similar to the RR of 0.77 (0.75–0.79) with LDLC reduction (70). All drugs including fibrates (except for CETP inhibitors) fitted the regression line to predict RR reduction when

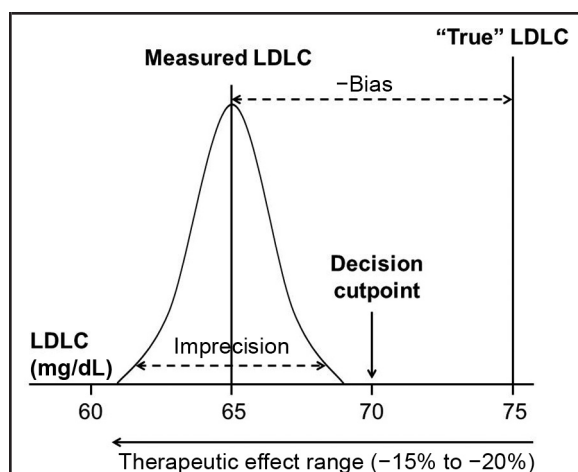


Fig. 2. Example of potential confounding of negative measurement bias and between-laboratory imprecision in the clinical interpretation of LDLC concentration.

In this example, LDLC is measured with a certain assay method in different laboratories and with the reference method β -quantification ("true" LDLC) in a patient with very high risk (desirable LDLC concentration, <70 mg/dL; 1.8 mmol/L). With LDLC measured in laboratories using this type of assay, it will be falsely concluded that the patient is at goal and can be excluded from LDLC-lowering therapy. The negative bias of this assay method compared with β -quantification is in the same range as can be observed with a low-potency therapeutic effect, e.g., ezetimibe, or nutraceutical effect. To convert LDLC to mmol/L, divide value in mg/dL by 38.6.

non-HDLc was used, but not when LDLc was used (70). In the REVEAL study, the CETP inhibitor anacetrapib fitted the regression line when non-HDLc reduction was applied (68).

These findings support the use of non-HDLc as a target of therapy as recommended by guidelines (1–4). The recommended targets for non-HDLc typically are arbitrarily set 30 mg/dL (0.8 mmol/L) higher than LDLc targets; this value assumes that the "normal" VLDLc level associated with the fasting TG cutpoint of 150 mg/dL (1.7 mmol/L) is 30 mg/dL (0.8 mmol/L), as estimated by the Friedewald formula ($TG/5$ in mg/dL or $TG/2.2$ in mmol/L). Thus, a non-HDLc goal of <100 mg/dL (2.5 mmol/L) is equivalent to an LDLc goal of <70 mg/dL (1.8 mmol/L) in very high-risk subjects (19) (Table 3). For very low or very high ranges of non-HDLc, simple addition of a fixed term of 30 mg/dL (0.8 mmol/L) is not appropriate. It may be more appropriate to use a multiplier of 1.3, so that an LDLc of 2 mmol/L is equivalent to a non-HDLc of 2.6 mmol/L (100 mg/dL), and a low LDLc of 1 mmol/L is equivalent to a non-HDLc closer to 1.3 mmol/L (50 mg/dL) rather than

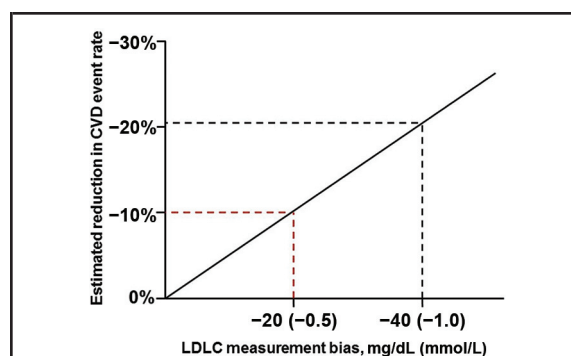


Fig. 3. Potential confounding of biased LDLC measurement in the estimation of CVD risk reduction.

Estimated CVD risk reduction in study populations is based on the Cholesterol Treatment Trialists' metaanalysis of LDLc-lowering trials. Measurement bias up to -0.5 mmol/L (-20 mg/dL) can be falsely interpreted as a 10% CVD risk reduction when discordant LDLc assay methods are used from baseline to on-treatment measurements.

1.8 mmol/L (70 mg/dL). This is in line with the previously proposed adjustable factor for VLDLc estimation based on TG and non-HDLc concentrations (53, 54).

Non-HDLc can be obtained in the nonfasting state and does not require TG to be <400 mg/dL (4.5 mmol/L) (19). Therefore, it is a useful alternative to cLDLc when the Friedewald equation is invalid, essentially making the need for a dLDLc assay obsolete. Although non-HDLc is not dependent on TG variability, dHDLc measurement errors in hypertriglyceridemic samples still affect the calculation of non-HDLc. Accuracy-based EQA surveys using the different dHDLc assays in the calculation revealed median bias of $<10\%$ for all methods, as compared with the expected non-HDLc value based on TC and HDLc reference measurements, although the range of individual laboratory biases reported was broad in moderate to severe hypertriglyceridemic serum samples (47, 48). Therefore, not only calculated LDLc but also non-HDLc values must be interpreted with caution when making treatment decisions for individuals with dyslipidemia (Table 5). However, in samples from those with moderate hypertriglyceridemia in the 200 to 399 mg/dL (2.3–4.5 mmol/L) range, non-HDLc calculated with different dHDLc assays showed much better concordance with CVD risk classification according to NCEP non-HDLc cutpoints (overall misclassification range, 0%–14%) than either dLDLc or cLDLc test results according to LDLc cutpoints (overall misclassification range, 7%–37%) (66). Consequently, under conditions of high TG when cLDLc is likely to be inaccurate, non-HDLc can be calculated. The

benefit of non-HDLc over LDLc may arguably be cost, as it can be calculated at no additional expense above conventional lipid testing.

LDL PARTICLE NUMBER

LDL can be subfractionated by NMR spectroscopy, density-gradient ultracentrifugation, electrophoresis, ion-mobility analysis, or chromatography (71, 72). NMR spectroscopy is by far the least labor-intensive method (72). NMR spectroscopy provides rapid quantification of size and concentration (LDLP) of LDL particles and various lipoprotein subclasses, including VLDL, IDL, LDL, and HDL; Lp(a) is typically included in the measured LDL fraction with NMR. Lipoproteins are analyzed by NMR according to the spectral signals produced by the terminal methyl groups contained within the lipid particles. The number of methyl groups present in TG, cholesterol, and phospholipids is consistent for particles of a given size, allowing for translation into particle concentration (72). Nonetheless, this technology has not been exhaustively standardized to account for the wide compositional variability occurring in neutral core lipids in different dyslipidemic conditions; therefore, caution is warranted in data interpretation under these conditions. NMR lipoprotein analyses have been evaluated against other existing technologies to determine numbers, size, and subclasses of lipoprotein particles, but the results agree poorly with each other, in part because the various techniques measure different physical properties (71, 73). However, even different NMR methods yield discrepant results, as the algorithms to decipher the NMR signals are proprietary developments of each provider (74). Therefore, standardization of NMR spectral analysis is urgently needed, as well as harmonization to a unique reference material to allow comparability of NMR data with other subfractionation techniques (72).

Regardless, multiple large prospective cohort studies that have monitored clinical outcomes, including EPIC-Norfolk, Framingham Offspring, Multi-Ethnic Study of Atherosclerosis (MESA), and Women's Health Studies have demonstrated that LDLP is superior to LDLc in predicting CVD, consistent with the fact that many individuals with atherogenic dyslipidemia have increased numbers of LDLP without having high LDLc (75–79); in this situation, patients often have higher concentrations of TG-rich lipoproteins, which may explain the higher CVD risk rather than high LDLP numbers per se. LDLP also appears to be a better indicator of subclinical CVD because it associates more strongly with coronary artery calcium and carotid intima-media thickness than LDLc or non-HDLc (80). Whereas earlier studies emphasized the atherogenicity of small LDL particles, we now know that all LDL particles are atherogenic (81), as evidenced by patients with familial hypercholesterolemia who display a predominance of large, buoyant LDL par-

ticles and early atherosclerosis (82). Thus, the primary focus of patient treatment should remain the reduction of the number (concentration) of LDL particles, without efforts to distinguish between large and/or small LDL particles. Indeed, a review of 24 studies that reported relationships between LDL subfractions and cardiovascular outcomes concluded that higher LDLP but not LDL size was consistently associated with increased risk of CVD events (83).

A major impediment to LDLP testing is its limited availability outside the US, although it is offered by some larger reference laboratories and a small automated NMR analyzer (Vantera) has been developed by Liposcience (now LabCorp) for high-throughput clinical laboratory testing (84). Another potential barrier is its cost, which is about twice that of a standard lipid panel, although it does provide additional information on other lipoprotein fractions. NMR quantification of VLDL particle concentration (VLDLP) revealed that the smallest remnant subclass concentration was associated with a 68% per SD (HR, 1.68; 95% CI, 1.28–2.22) increase in residual risk among statin-treated patients with low LDLc (85). The use of LDLP and VLDLP subclass monitoring as an additional treatment target beyond LDLc needs more widely accessible and standardized assays in clinical laboratories before it can be recommended for routine clinical practice.

APOLIPOPROTEIN B

ApoB is the structural protein for all non-HDL lipoproteins and modulates the centrifugal and centripetal transportation of lipoproteins with reference to liver, intestine, and peripheral tissues (36). There are 2 isoforms of apoB: the full-length form of apoB, apoB100 with a molecular mass of 550 kDa, the major isoform synthesized in hepatocytes and found in VLDL, IDL, LDL, and Lp(a); and a truncated form, apoB48, with a molecular mass of 265 kDa (48% of the molecular weight of apoB100), that is synthesized in intestinal enterocytes and is the structural protein of chylomicrons and chylomicron remnants (36). ApoB100, but not apoB48, contains the ligand that binds to the LDL receptor. Because each atherogenic particle contains 1 molecule of apoB, concentrations of apoB are considered to be a direct measure of the total number of atherogenic lipoproteins in the circulation (36).

Because of its relatively high abundance, apoB100 can be readily measured by immunoassays. Immunoturbidimetric and immunonephelometric assays are commercially available on commonly used automated systems from several manufacturers. Most immunoassays detect both apoB100 and apoB48, depending on the specificity of the antibodies used, which are typically generated against apoB100. In fasting samples, >90% of apoB in plasma is apoB100. Because of the longer half-

life of LDL (3 or 4 days) compared with VLDL (3 or 4 h), almost all of it is associated with LDL when TG concentrations are <200 mg/dL (2.3 mmol/L) (36). Therefore, at low TG concentrations, the measurement of apoB is essentially an estimate of LDL–apoB concentration or LDLP. Simple apoB testing obviates the need for more expensive technologies such as NMR spectroscopy to measure LDLP, although apoB cannot substitute for NMR-based particle size measurements (38). LDLP can be directly compared with apoB100 measurements by adding VLDLP to LDLP and converting nanomoles per liter (nmol/L) to mass units (mg/dL) and by multiplication by the factor 0.055 based on the molecular weight of apoB100 (550 kDa) (38). This conversion recognizes that both apoB100 and LDLP measurements include IDL and Lp(a), in addition to LDL (38).

From an analytical viewpoint, apoB100 is a clearly defined protein. Its measurement can be standardized because of the availability of serum-based IFCC/WHO SP3–08 secondary reference material, value-assigned using immunonephelometry and the primary reference material—a stable LDL fraction prepared by ultracentrifugation (density, 1.030–1.050 g/mL) that excludes IDL and Lp(a) (86). ApoB100 measurements are easily automated and yield more accuracy compared with direct assays for LDLC or HDLC, although concerns about the accuracy of apoB immunoassays still exist by comparison with apoB concentrations derived from NMR and vertical autoprofile ultracentrifugation in subjects with hypertriglyceridemia (87). Uniform calibration has reduced between-laboratory variability (CV) of apoB immunoassays from $>19\%$ to approximately 7% to 9% (88). In the EQA program in the Netherlands, 28% of participating laboratories exceeded the NCEP bias recommendation for apoB ($\leq 6\%$), whereas 68% exceeded the recommendation for LDLC ($\leq 4\%$) (88). Notwithstanding the availability of internationally recognized reference systems, more efforts from in vitro–diagnostic industry and laboratory professionals have the potential to further improve apoB standardization and precision to approximately 2% to 4% (88). Far higher than the maximum allowed TG concentration of 4.5 mmol/L (400 mg/dL) for cLDLC, most turbidimetric and nephelometric apoB100 immunoassays allow TG up to at least 10 mmol/L (880 mg/dL) without interference; a TG concentration above this limit in nonfasting blood samples is seen in only approximately 0.1% of individuals in the general population (24).

More recently, LC-MS/MS-based quantification of apolipoproteins has been introduced (89, 90). Automated mass spectrometry enables precise measurements of apoB100 (total CV, 2.5%–4%) that are interchangeable with immunoturbidimetric assays in both normotriglyceridemic and hypertriglyceridemic sera with TG ≤ 20 mmol/L (1770 mg/dL) (90). However, the

clinical applicability and throughput of these LC-MS/MS assays are limited by the complexity and processing time of the sample preparation that is typically performed before protein measurement. With improvements in the work flow using automation and shorter chromatographic run times, LC-MS/MS protein assays may be translated to large clinical studies and eventually to regular clinical laboratory operations. Another advantage of LC-MS/MS is that it enables the simultaneous (multiplexed) measurement of multiple apolipoproteins in a single run of the assay, thus making it possible to achieve a complete apolipoprotein profile in the patient. In the case of VLDL-associated apolipoproteins apoC-I, apoC-II, apoC-III, and apoE, standardized clinical immunoassays are generally not available, which impedes discovery of pathophysiological clues (e.g., apoC-II deficiencies or apoC-III increases) for adequate diagnosis and management of dyslipidemia. Mass spectrometry-based proteomics revealed strong associations of VLDL-associated apolipoproteins with incident CVD in the prospective Bruneck Study population, which supports the concept of targeting TG-rich lipoproteins to reduce risk of CVD (91).

The use of traceable serum-based calibrators will improve interlaboratory reproducibility of LC-MS/MS methods and may contribute to a more rapid transition of biomarker discovery to clinical utility to identify personalized treatment opportunities for dyslipidemia in CVD patients. For this purpose, the IFCC Scientific Division established a working group in 2017 to achieve standardization of a panel of the clinically relevant serum apolipoproteins A-I, B, C-I, C-II, C-III, E, and apo(a), and to develop an LC-MS/MS-based reference measurement system for the aforementioned analytes that are unaffected by genetic variants, posttranslational modifications, and other factors.

PREANALYTICAL ADVANTAGES OF apoB

In addition to analytical variability, biological within-subject (intraindividual) variability should be considered. The average biological variability (intraindividual CV) of apoB is 6% to 7% (range, 3%–12%), making repeated measurements of apoB in the single patient more reliable than calculation of non-HDLc because the latter includes the biological variations of both TC (6% CV, range 2%–12%) and HDLC (7% CV, range 2%–14%) (92). The intraindividual CV of TG (average, 28%) is a major contributor to the overall variability of cLDLC and ranges widely to as high as 75% (92). This presents a major advantage when testing apoB or non-HDLc, which are not affected by biological variation in TG.

Similar to non-HDLc, fasting is never required for apoB measurement. Although each chylomicron and chylomicron remnant particle contains 1 molecule of apoB48, this does not present a problem in the apoB

immunoassay. Except for type III hyperlipoproteinemia (dysbetalipoproteinemia), there are so few chylomicron and chylomicron remnant particles, even compared with VLDL particles, that they do not appreciably influence total apoB concentrations (13, 36). Even at peak postprandial concentrations, the number of chylomicron–apoB48 particles in healthy individuals is usually <1% and the number of VLDL–apoB100 particles is <10% of the number of LDL–apoB100 particles (36). Thus, even if the number of chylomicrons increases 5- to 10-fold, no substantial changes will occur in total apoB concentration. In contrast, the contribution of Lp(a)–apoB100 to apoB concentration can be substantial (>10%) among individuals with very high Lp(a) >100 mg/dL (93). Considering that apoB100 contributes approximately 16% of Lp(a) mass, Lp(a)-corrected apoB concentration can be estimated as $\text{apoB (mg/dL)} = [\text{Lp(a) (mg/dL)} \times 0.16]$ (93).

Consensus-based recommendation. Non-HDLC and apoB tests are more accurate than dLDLC and cLDLC, especially for measurements in samples that are hypertriglyceridemic, nonfasting, or at low LDLC concentrations. Non-HDLC offers the advantage of adding Remnant-C to LDLC, independent of TG variability (CBR6), but is still compromised by nonspecificity bias of dHDL used in the calculation. ApoB has the potential to meet analytical performance criteria in terms of accuracy, standardization, availability on common laboratory instruments, and relatively low cost, and can be used to estimate numbers (concentrations) of LDLP plus remnant-P (VLDLP) and Lp(a) particle numbers, but not size (CBR7) (38). Multiplex LC-MS/MS apolipoprotein profiles or NMR-based LDLP and VLDLP subclass numbers and size are promising approaches for use in personalized (precision) medicine for cardiovascular risk management but still need standardization if laboratory data from several epidemiological studies and clinical trials are pooled for evaluation of clinical performance of the tests. Furthermore, wide accessibility is critical to enable clinical use.

Should Non-HDLC or apoB Replace LDLC Tests?

OBSERVATIONAL STUDIES IN GENERAL POPULATIONS

Studies have reported inconsistent findings as to whether non-HDLC or apoB identifies CVD risk more effectively than traditional LDLC testing. A metaanalysis of 12 epidemiological studies including 233 455 individuals concluded that apoB, with an RR of 1.43 (95% CI, 1.35–1.51) per 1-SD increment, was superior to non-HDLC (1.34; 1.24–1.44) and LDLC (1.25; 1.18–1.33) in the association with future fatal or nonfatal CVD events ($P < 0.001$) (94). In 10% of women and 20% of men in

the Copenhagen City Heart Study ($n = 9231$) included in this metaanalysis, apoB predicted a higher risk than cLDLC-related risk for developing CHD ($P < 0.01$); these individuals had higher TG and an optimal cLDLC concentration (95). However, in 4679 MESA study participants, associations of lipoprotein particle measures (i.e., apoB and LDLP) with CHD were attenuated after adjustment for standard lipid variables, indicating that their measurement does not detect risk that is unaccounted for by the standard lipid panel (96).

A large-scale metaanalysis performed by the Emerging Risk Factors Collaboration group on data of 302 430 patients (involving 12 785 CHD cases) from 68 prospective studies showed that adjusted HRs for CHD risk for 1-SD higher values were 1.50 (95% CI, 1.39–1.61) for non-HDLC and 0.99 (0.94–1.05) for TG after adjustment for non-HDLC and HDLC (97). For a subset of 44 234 participants from 8 studies with measured dLDLC, HRs were similar for non-HDLC and dLDLC, 1.42 (1.06–1.91) and 1.38 (1.09–1.73), respectively. HRs were at least as strong in nonfasting participants than in those who did fast (97). The same group performed another metaanalysis of 26 observational studies (139 581 participants, 12 234 events) that also had information on apolipoproteins and found similar prognostic values: HRs per 1-SD increase for apoB and non-HDLC were 1.24 (1.19–1.29) and 1.27 (1.22–1.33), respectively (98). Replacement of information on TC with various lipid parameters, including non-HDLC and apoB, did not improve CVD prediction (98).

A systematic review of 9 relevant studies by the Laboratory Medicine Best Practices method indicated that addition of apoB to standard risk factors (RR = 1.31; 95% CI, 1.22–1.40) resulted in significant improvement in long-term CVD risk assessment (99); however, because there were an insufficient number of studies, no conclusion was made for the effectiveness of non-HDLC in predicting CVD events (99).

INTERVENTIONAL STUDIES

On-treatment apoB adds prognostic information to LDLC and even to non-HDLC in some but not all primary prevention and secondary prevention trials. A metaanalysis of on-treatment data from 38 153 participants in 8 randomized controlled statin trials revealed adjusted HRs per 1-SD higher of 1.13 (95% CI, 1.10–1.17) for LDLC, 1.16 (1.12–1.19) for non-HDLC, and 1.14 (1.11–1.18) for apoB (100). All 3 were generally similar in magnitude of CVD residual risk, although only the small difference between LDLC and non-HDLC was statistically significant ($P = 0.002$), whereas the difference between LDLC and apoB was not significant (100). Changes in non-HDLC also explained a larger proportion (64%) of the statin-induced atheroprotective effect than did LDLC (50%) or apoB (54%) (100). It should

be noted that most of the studies included in the metaanalysis used heparin-Mn²⁺ precipitation for HDLC measurement, which differs from direct assays mostly used nowadays; therefore, these results based on non-HDLC may not translate to current clinical laboratory practice.

A larger metaanalysis of 25 trials conducted in 131 134 patients including 12 on statin, 4 on fibrates, 5 on niacin, and 2 on simvastatin-ezetimibe, came to similar conclusions using Bayesian random-effect analysis: Non-HDLC was slightly superior to apoB for prediction of CHD (Bayes factor, 1.45) and CVD (Bayes factor, 2.07) (101). Only the combination of apoB with non-HDLC or LDLC slightly improved CVD risk prediction (Bayes factor, 1.13). Combining the 25 trials, each 10-mg/dL decrease in apoB was associated with a 9% decrease in CHD, no decrease in stroke, and a 6% decrease in major CVD risk (101). In the JUPITER trial in adults without diabetes or CVD, with baseline cLDLC <130 mg/dL (3.4 mmol/L) and C-reactive protein concentrations ≥ 2 mg/L, standardized HRs did not differ for on-statin cLDLC, non-HDLC, and apoB: 1.31 (95% CI, 1.09–1.56), 1.25 (1.04–1.50), and 1.27 (1.06–1.57), respectively (102). Interestingly, in the JUPITER trial, baseline apoB, LDLP, VLDLP, Remnant-C, and non-HDLC were all associated with increased CVD risk, whereas cLDLC was not (85).

In another metaanalysis, the mean CHD risk reduction per SD decrease was higher for apoB (24%; 95% CI, 19%–29%) compared with 20% (16%–24%) for LDLC and 20% (15%–25%) for non-HDLC across 7 major statin trials (103). Within each trial, risk reduction per change in apoB averaged 22% (12%–31%) greater than changes in LDLC and 24% (22%–26%) greater than changes in non-HDLC ($P < 0.001$). In the same metaanalysis, 1-SD decreases to equivalent target concentrations of LDLC (–42%), non-HDLC (–41%), and apoB (–42%) would yield expected risk reductions of 30%, 32%, and 39%, respectively, suggesting that benefit would be greater if therapy were targeted to apoB rather than to LDLC or non-HDLC (103).

Consensus-based recommendation. Metaanalyses among study populations suggest that the clinical performance of non-HDLC and apoB, although superior to LDLC in some studies, is, on average, comparable with LDLC to predict risk. However, in a subset of individuals in whom apoB or non-HDLC is high despite a normal or low LDLC, CVD risk tracks with apoB or non-HDLC (CBR8).

Should Non-HDLC or apoB Be Used as “Add-on” Tests to LDLC?

Although there is high overall correlation and equivalent predictive power in large population-based studies and

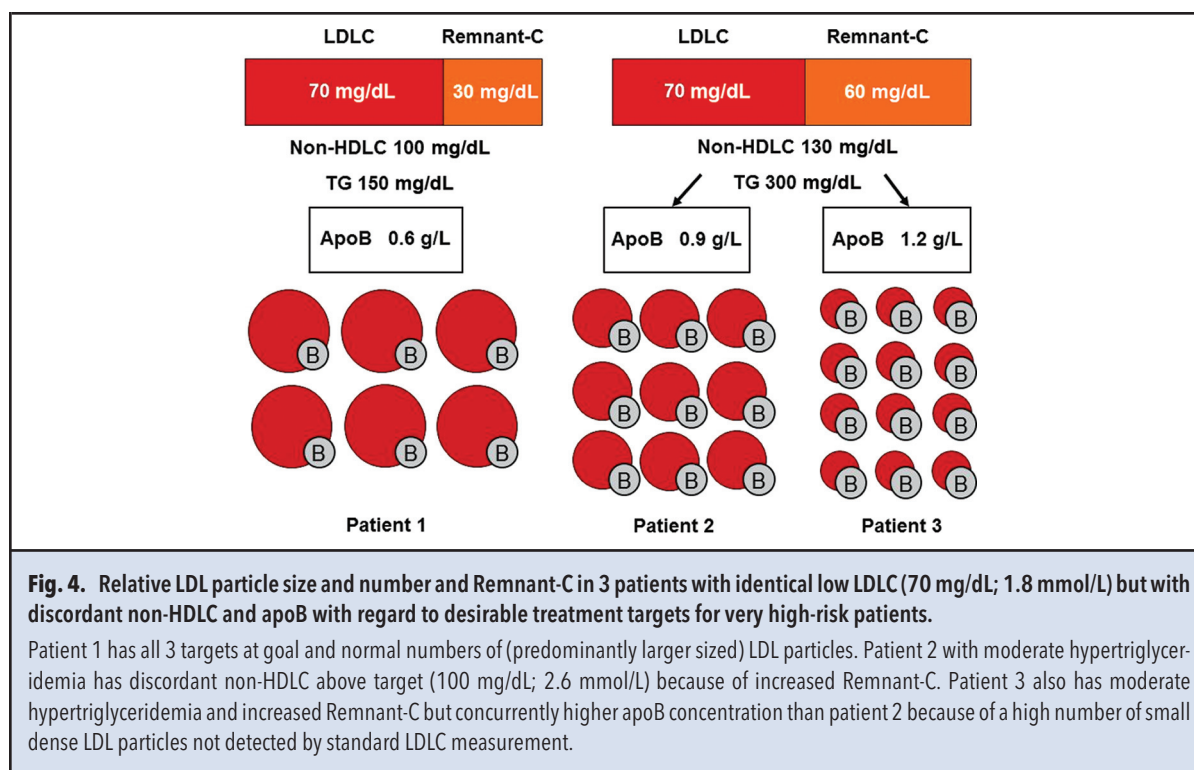
trials, data from concordance/discordance analyses reveal that the addition of non-HDLC or apoB to LDLC has the potential to improve risk prediction by identifying more high-risk individuals in a personalized approach. Discordances (1 normal, the other high) exist between LDLC and non-HDLC, LDLC and apoB, and non-HDLC and apoB, and may be present in up to 25% of the general population (Fig. 4).

LDLC VS NON-HDLC

Despite the correlation between non-HDLC and either cLDLC or dLDLC measurements in study populations, it is evident that non-HDLC and LDLC do not consistently provide the same clinical information in individual patients. Among individuals with the same non-HDLC value, there can be a 40- to 60-mg/dL (1.0–1.6 mmol/L) difference in cLDLC, depending on the range of TG concentrations between 100 and 300 mg/dL (1.1–3.4 mmol/L) (104). The correlation between non-HDLC and dLDLC significantly decreases with increasing TG concentrations: In a study of 1590 patients, the percentage of patients with non-HDLC concentration above the guideline-based cutpoint of 130 mg/dL (3.6 mmol/L), despite having dLDLC concentration on target at ≤ 100 mg/dL (2.6 mmol/L), increased significantly with increasing TG (105). Replacing dLDLC with non-HDLC as a therapeutic target in hypertriglyceridemic patients almost doubled the number of patients requiring treatment (105).

In a large database of lipid profiles of 1 310 440 patients, non-HDLC reclassified a significant proportion of patients to a higher guideline-based treatment category compared with cLDLC, especially at low LDLC in the treatment range and at TG ≥ 150 mg/dL (1.7 mmol/L) (106). Of patients with LDLC <70 mg/dL (1.8 mmol/L), 15% had non-HDLC ≥ 100 mg/dL (2.6 mmol/L) and 22% had this value if TG concentrations were 150 to 199 mg/dL (1.7–2.3 mmol/L) concurrently (106). Similarly, of patients with LDLC concentrations between 70 and 99 mg/dL (1.8 and 2.6 mmol/L), 12% had non-HDLC ≥ 130 mg/dL (3.4 mmol/L) and 17% had this value if TG concentrations were concurrently 150 to 199 mg/dL (1.7–2.3 mmol/L), and discordance between LDLC and non-HDLC percentiles increased at lower LDLC and higher TG concentrations (106).

The potential clinical implications of discordant LDLC and non-HDLC are most evident at normal or low LDLC concentrations. Among apparently healthy individuals with cLDLC <100 mg/dL (2.6 mmol/L) in the EPIC-Norfolk prospective population study ($n = 21\,448$), those with non-HDLC >130 mg/dL (3.4 mmol/L) had an HR for future CHD of 1.84 (95% CI, 1.12–3.04) compared with those with non-HDLC <130 mg/dL (3.4 mmol/L) (107). The risk associated with a 1-SD increase of non-HDLC (HR = 1.54; 95%



CI, 1.35–1.74) was higher than the risk associated with a 1-SD increase of cLDLC (HR = 1.22; 1.17–1.27) or TG (HR = 1.14; 1.09–1.19). The higher risk associated with increased non-HDLc was present at any category of LDLc concentrations, and particularly at low LDLc concentrations, whereas LDLc did not provide any additional risk to non-HDLc for CHD (107). This notion implies that there is no reason to use non-HDLc as an “add-on” test: If concordant, it performs equally as well as LDLc. If discordant, it performs better. This implies that the net result is an overall better performance of non-HDLc compared with LDLc.

The same reflection is applicable to the metaanalysis by Boekholdt et al. of 8 statin trials (100). In this analysis, HRs for major CVD risk were calculated for 4 categories of 38 153 treated patients based on whether they reached the LDLc target of 100 mg/dL (2.6 mmol/L) and the non-HDLc target of 130 mg/dL (3.4 mmol/L) (100). Statin-treated patients reaching the non-HDLc target but not the LDLc target had an HR of 1.01 (95% CI, 0.92–1.12) compared with those reaching both targets. Patients reaching the LDLc target but not the non-HDLc target had an HR of 1.32 (1.17–1.50) (100).

These data suggest that calculation of non-HDLc is at least equally good at predicting CHD as compared with measurement or calculation of LDLc in the overall population, and may be superior to LDLc if discordant in individuals with high TG because it includes VLDLC

(= Remnant-C). The only concern is the selection of the cutoff value: It may be the more sensitive risk cutpoint for non-HDLc as compared with LDLc rather than the biomarker that makes the difference. Guideline-based non-HDLc cutpoints have been arbitrarily defined by consensus of expert groups and societies, based on the assumption that a normal VLDLC concentration exists when TG are <150 mg/dL (1.7 mmol/L), which is <30 mg/dL (0.8 mmol/L) as estimated by the Friedewald formula. Lowering non-HDLc cutpoints leads to upward reclassification of patients (if the goal is to reduce undertreatment), and higher cutpoints lead to downward reclassification (if the goal is to reduce overtreatment). Risk cutoff values need to be validated for diagnostic performance. For present purposes, the combination of non-HDLc with cLDLC data seems an appropriate strategy to guide therapy. This may compensate for the underestimation or overestimation of LDLc in terms of clinical decision-making, given the uncertainty of the measurement or calculation when LDLc approaches 70 mg/dL (1.8 mmol/L). Utilizing non-HDLc, at no additional expense, appears to be preferable to dLDLC when cLDLC is not available because of an invalid Friedewald equation (CBR9). However, the compromised accuracy of dHDLc assays in samples with hypertriglyceridemia reduces the benefit in reporting non-HDLc in some individuals (48).

LDLC VS apoB

The implication of discordant apoB vs LDLC is most evident in patients with atherogenic dyslipidemias, who present with “normal” concentrations of TC and LDLC—a profile that is especially prevalent among individuals with the metabolic syndrome or insulin resistance and in those taking medications, such as statins, that reduce LDLC more than apoB (108). In 1522 individuals in the Insulin Resistance Atherosclerosis Study, increased apoB with normal cLDLC in each NCEP Adult Treatment Panel III risk category was more strongly associated with risk factors including abdominal obesity, dyslipidemia, hyperinsulinemia, and thrombosis than increased cLDLC with normal apoB (109), consistent with the notion that cardiovascular risk is more directly related to the number of apoB-containing particles (reflected by apoB measurement) than to the cholesterol content of lipoproteins (81). Therefore, risk may be underestimated on the basis of TC and LDLC alone in subjects with predominant small, cholesterol-depleted LDL particles, whereas increased apoB concentration helps identify these high-risk individuals who would have otherwise been overlooked because of their “optimal” LDLC, a situation estimated to apply to approximately 30% of the population (108).

For example, when evaluating a patient with diabetes and an LDLC of 90 mg/dL (2.3 mmol/L), it is often concluded that they are at goal because their LDLC is <100 mg/dL (2.6 mmol/L) (1). However, this level represents higher numbers of cholesterol-poor LDL particles and puts the patient at higher risk compared with a person with an LDLC of 90 mg/dL (2.3 mmol/L) but without atherogenic dyslipidemia and associated insulin resistance. Another problem is that these patients tend to have increased concentrations of TG-rich lipoproteins, and by the Friedewald equation, their LDLC will be underestimated (57, 58).

The discordance analysis of the Women’s Health Study (110) represents a comprehensive comparison of atherogenic lipoprotein markers. Despite significant LDLC correlations with non-HDLc, apoB, and LDLP observed in the 27 533 study participants, prevalence of LDLC discordance as defined by median cutpoints was 12%, 19%, and 24% for non-HDLc, apoB, and LDLP, respectively. Among women with dLDLC less than the median (121 mg/dL; 3.1 mmol/L), CHD risk was underestimated 3-fold for those with discordant (the median or more) non-HDLc (HR = 2.92; 95% CI, 2.33–3.67), apoB (HR = 2.48; 2.01–3.07), or LDLP (HR = 2.32; 1.88–2.85) compared with women with concordant concentrations; these individuals had increased TG, low HDLC, and smaller LDL particles that were cholesterol-depleted (110). Conversely, among women with dLDLC equal to the median or more, risk was overestimated 3-fold for those with discordant (less than the

median) non-HDLc (HR = 0.40; 0.29–0.57), apoB (HR = 0.34; 0.26–0.46), or LDL-P (HR = 0.42; 0.33–0.53); these individuals had increased LDLC because their LDL particles were larger and more cholesterol-enriched, despite having fewer overall numbers of LDLP or apoB (110).

Additional analysis with cLDLC instead of dLDLC revealed that, among women with cLDLC below median, HRs for discordant apoB or LDLP were increased (110). In a recent discordance analysis among 2794 young adults, high apoB and low cLDLC or non-HDLc discordance was associated with higher odds of developing coronary artery calcium in midlife: adjusted odds ratios [OR (95% CI)] were 1.55 (1.10–2.18) and 1.45 (1.01–2.09), respectively (111). Discordance analyses of cLDLC vs LDLP in the Framingham and MESA studies both favored LDLP over cLDLC among discordant individuals (75, 79).

These data suggest that for most individuals with concordant values of LDLC and an alternative measure (non-HDLc, apoB, or LDLP), the clinical performance of these measures is similar. However, among the subgroup of individuals (up to 25% of the Women’s Health Study population) with discordance of LDLC with alternative measure, risk may be overestimated or underestimated when one relies on LDLC alone (110). If apoB performs either equally well (if concordant) or better (if discordant) than LDLC, the 2-step procedure is redundant, and apoB could be the single test rather than an add-on test.

NON-HDLc VS apoB

Non-HDLc is not a clinically accurate surrogate for apoB because the 2 markers represent different measures. Non-HDLc and apoB are highly correlated in large population-based studies but only moderately concordant between individuals (108). At any non-HDLc value, there is considerable variation in apoB concentrations ranging from far above to far below the desirable values (112). Such discordance between non-HDLc and apoB is even more pronounced in patients with hypertriglyceridemia, particularly familial combined hyperlipidemia, which is characterized by hyper-apoB (37), and dysbetalipoproteinemia owing to the apoE2/2 genotype, which typically presents with low apoB concentration (and positively discordant non-HDLc with regard to apoB) because the β -VLDL remnants are not processed to LDL particles (37). None of those dyslipidemias can be characterized with non-HDLc (112).

Beyond LDLC, non-HDLc estimates the impact of 2 closely related metabolic biomarkers of atherogenic dyslipidemia, namely, (VLDL) remnant cholesterol and LDL particle number, but it does not accurately measure the latter. If LDL particles are cholesterol-depleted, as observed in atherogenic dyslipidemia, LDLC and non-

HDLC will underestimate the risk because of high LDL particle numbers (and consequently, discordant high apoB concentration) (112). Also, the mass of cholesterol within VLDL particles is highly variable. VLDL- and IDL-apoB100 make up a relatively small fraction (<10%) of total plasma apoB, whereas VLDLC can easily range from 10% to 25% or more of non-HDLC, with the result that there is much greater variance in VLDLC as a percentage of non-HDLC than there is of VLDL-apoB100 as a percentage of total apoB (108).

Discordance analysis of apoB vs non-HDLC in the INTERHEART case-control study demonstrated that apoB is not equivalent to non-HDLC in predicting CVD risk (113). The OR for cases to controls with a “non-HDLC>apoB phenotype” (cholesterol-enriched apoB particles) was 0.72 (95% CI, 0.67–0.77), indicating risk was less than the reference concordant group, whereas the OR for the “non-HDLC<apoB phenotype” (cholesterol-depleted apoB particles) was 1.58 (1.38–1.58), indicating risk was significantly greater than the concordant group (113).

In the Insulin Resistance Atherosclerosis Study (n = 1522), despite lower LDLC, the hyper-apoB group with normal non-HDLC had increased risk indicated by greater waist circumference, hyperinsulinemia, and lower insulin sensitivity (114). ApoB was more closely associated with central adiposity, insulin resistance, and inflammation than non-HDLC, suggesting that apoB is a better risk parameter than non-HDLC for identifying a subgroup of individuals with or without metabolic syndrome with increased cardiovascular risk (114). Once apoB concentrations are increased, then CHD risk can be considered high and non-HDLC yields negligible additional risk prediction.

Among 18 225 men in the Health Professionals Follow-up Study, non-HDLC and apoB were both strong predictors of CHD, more so than LDLC (115). In this male cohort, the RR of CHD (top vs bottom quintile) was 3.01 (95% CI, 1.81–5.00) for apoB, 2.76 (1.66–4.58) for non-HDLC, and 1.81 (1.12–2.93) for dLDLC. When non-HDLC and dLDLC were mutually adjusted, only non-HDLC was predictive of CHD. When non-HDLC and apoB were mutually adjusted, only apoB was predictive. Within each tertile of non-HDLC, the risk of CHD increased with increasing tertiles of apoB, whereas within each tertile of apoB, the risk did not increase by tertiles of non-HDLC. Values for TG concentrations added significant information to non-HDLC but not to apoB for CHD risk prediction (115).

In the Women's Health Study (n = 27 533), discordance among apoB, LDLP, and non-HDLC occurred in up to 20% of women (116). Women with discordant high particle concentration measured by apoB and LDLP were more likely to have metabolic syndrome and diabetes. Women with high particle concentration relative to

non-HDLC had increased CHD risk: Age-adjusted HR was 1.77 (95% CI, 1.56–2.00) for apoB and 1.70 (1.50–1.92) for LDLP. After adjustment for clinical risk factors including metabolic syndrome, these risks attenuated to 1.22 (1.07–1.39) for apoB and 1.13 (0.99–1.29) for LDLP. Importantly, most of these individuals with discordant high apoB or LDLP would be missed (deemed very low risk) by global risk algorithms (2). Discordant low apoB or LDLP relative to non-HDLC was not associated with lower risk (116).

The relative merits of apoB vs non-HDLC have been a point of ongoing debate and controversy. Both offer the practical benefits of assessment independent of the prandial state. Although apoB measurement comes at an extra cost, the findings from discordance analyses support our consensus that in certain patients the number of atherogenic lipoprotein particles measured by apoB (or LDLP) is more predictive of development of CHD than the cholesterol carried by these particles, measured by non-HDLC. It should be noted, however, that apoB and LDLP measurements may not always be equivalent in their ability to predict CVD risk. A metaanalysis in 2013 including 25 clinical studies revealed 21% discordance of apoB immunoassays and NMR-based LDLP measurements in their associations with diverse clinical outcomes, and the strength of association (as indicated by OR, RR, and HR) was more often higher for LDLP than it was for apoB (38).

ON-TREATMENT DISCORDANCES

The reduction of LDLC, non-HDLC, and apoB concentrations achieved with statin therapy displays large intra-individual variation, even at very low LDLC <70 mg/dL (1.8 mmol/L), as observed in a metaanalysis of 8 statin trials (117). Statins lower LDLC and non-HDLC significantly more than they lower apoB or LDLP (118). This is because statins lower larger cholesterol-rich LDL particles proportionately more than they do for smaller cholesterol-poor LDL particles (119). A metaanalysis of 17 000 patients from 11 statin trials showed that LDLC was reduced by 43%, non-HDLC by 39%, and apoB by 33% (118). This necessarily results in an on-treatment LDLP concentration that, on average, is higher than would be anticipated from the concurrent LDLC or non-HDLC. However, high Lp(a) concentrations in some patients will also help explain why apoB and LDLP are reduced less than LDLC or non-HDLC. The average on-treatment LDLC is reduced to the 20th percentile and non-HDLC to the 29th percentile, but apoB and LDLP decrease only to the 55th and 51st percentile of the population, respectively (120). This discordance points to substantial residual risk in many statin-treated patients and the opportunity for further benefit of LDL-lowering therapy that is lost if apoB or LDLP is not measured. In the AFCAPS/TexCAPS trial, on-treatment apoB maintained

its strong risk relationship with major coronary events after 1 year of lovastatin therapy, whereas LDLC lost statistical significance (121). Residual risk is also associated with on-treatment non-HDLc and more prominently with concentrations of smaller VLDLP, which may represent remnants of TG-rich lipoproteins that are insufficiently reduced by LDL-targeted statin dosages (85, 119, 122).

A large proportion of treated patients achieving their LDLc and even non-HDLc goals fail to meet their apoB target without more aggressive therapy (123–125). In the US National Health and Nutrition Examination Survey 2009–2010, 64% of statin-treated adults were at goal for LDLc and 63% were at goal for non-HDLc, but only 52% were at goal for apoB (126). Among those at goal for non-HDLc, 50% of those with CHD and 33% of other high-risk adults (including patients with diabetes and chronic kidney disease) were not at apoB goal (126). The presence of atherogenic dyslipidemia is associated with failure to meet all 3 targets of LDLc <70 mg/dL (1.8 mmol/L), non-HDLc <100 mg/dL (2.6 mmol/L), and apoB <0.8 g/L, such a nonachievement being found in a large proportion (one-third) of very high risk type 2 diabetes patients with on-statin LDLc <70 mg/dL (1.8 mmol/L) (127).

Discordances persist with increasing intensity of statin treatment. Combined analysis of 2 randomized secondary prevention trials with statins, TNT and IDEAL, showed that non-HDLc was reduced to the 30th percentile and apoB was reduced only to the 60th percentile of the low-intensity statin group (128). In the high-intensity statin group, non-HDLc was reduced to the 5th percentile, whereas apoB was reduced to only the 30th percentile (128). In the JUPITER trial of high-intensity statin, 46% of treated patients showed an LDLc reduction $\geq 50\%$, 28% showed non-HDLc reduction $\geq 50\%$, and only 18% showed apoB reduction $\geq 50\%$ (7). It remains to be demonstrated whether intensifying treatment strategies to further reduce undesirable apoB concentrations would yield an improvement in outcomes. A smaller change in apoB concentrations may not necessarily need to translate into smaller risk reduction. If 30% apoB decrease confers the same risk reduction as 50% LDLc decrease, it will not be a superior target. To test this, one would need to compare 2 interventions that lower apoB and LDLc disproportionately and assess the population attributable risks associated with the changes, i.e., the number (or proportion) of CVD cases that would not occur in a population if the risk factor were further lowered. In the JUPITER trial, similar relationships between percent reduction and treatment efficacy were observed in clinical outcome analyses focusing on LDLc, non-HDLc, or apoB (7).

Discordances in significant reductions in LDLc, non-HDLc, and apoB concentrations are also observed in anti-PCSK9 trials. Typically, PCSK9 inhibition causes average reductions of 60% to 65% in LDLc, 50% to 55%

reductions in non-HDLc, and 45% to 50% reductions in apoB from baseline depending on PCSK9 monotherapy or addition to moderate- or high-intensity statin (\pm ezetimibe) therapies in patients with hypercholesterolemia (129–132).

The reductions of LDLc, non-HDLc, and apoB obtained with anti-PCSK9 therapy are similar in patients with and without mixed hyperlipidemia, reflecting higher circulating concentrations of TG and remnant-like lipoproteins. Efficacy analysis of 3146 participants in 4 studies of evolocumab showed that the mean percentage change from baseline for patients with or without increased fasting TG >150 mg/dL (1.7 mmol/L) followed a similar pattern for LDLc (-67% and -65% vs placebo), non-HDLc (-53% and -54%), and apoB (-49% and -50%) (133). A similarly high proportion of evolocumab-treated NCEP III high-risk patients with and without increased TG achieved the LDLc target of <70 mg/dL (1.8 mmol/L) (82% vs 81%, respectively) and <100 mg/dL (2.6 mmol/L) (92% vs 92%, respectively). However, significantly more patients without increased TG achieved the apoB target of <0.8 g/L than those with increased TG (93% vs 85%). Additionally, significantly more patients without increased TG achieved the non-HDLc target of <100 mg/dL (2.6 mmol/L) than those with increased TG (85% vs 77%), suggesting that PCSK9 inhibition less efficiently reduces Remnant-C than LDLc in patients with hypertriglyceridemia (133).

Consensus-based recommendation. Discordance analysis of LDLc, non-HDLc, and apoB goals suggests opportunities to identify “hidden” CVD risk and to judge the adequacy of therapy. To reach the current proposed non-HDLc and apoB goals, novel therapies in addition to high-dose statins would inevitably be needed to further lower non-HDLc or apoB at very low concentrations of LDLc; however, the evidence base for this approach is still incomplete. The cutpoints for non-HDLc and apoB are arbitrarily defined by consensus cutoffs and need to be validated for diagnostic performance before they can be used as “add-on” tests to identify and treat additional persons at high risk. One would need to compare the reduction in CVD events achieved at each level of LDLc, non-HDLc, or apoB changes in the study data sets; otherwise, it is not clear whether the biomarker or the cutoff makes the difference.

For now, we recommend that every lipid profile report should add non-HDLc (CBR5). For those who determine TC, TG, and HDLc (and calculate dLDLc), this comes at no extra cost, as does calculated Remnant-C, which likewise can be reported free of charge. To improve patient comfort and compliance, there are advantages of this approach using nonfasting blood samples (19). When LDLc is unavailable owing to an invalid Friedewald equation (TG >400 mg/dL; 4.5 mmol/L), additional dLDLc measurement is not nec-

essary, and non-HDLc calculation can be used instead of cLDLc to evaluate therapeutic response (CBR9).

Which Is the Ideal Atherogenic Lipoprotein Test?

The traditional lipid profile of TC, TG, HDLc, and LDLc remains the primary approach for dyslipidemia diagnosis and CVD risk categorization. The position of LDLc is, however, challenged under treatment. The substantial residual risk that persists in LDLc-targeted therapies even at low LDLc <70 mg/dL (1.8 mmol/L) has fueled the debate about the cost-effectiveness of the clinical use of non-HDLc or apoB as an index of treatment efficacy (134). Thus, although the lipid profile will remain essential for initial diagnosis and risk categorization, one might suggest that follow-up of lipid-lowering therapy could be simplified and expenses reduced by calculation of non-HDLc (by only 2 measurements of TC and HDLc) or single measurement of apoB, without the need to fast and without regard to TG (134). Use of either non-HDLc or apoB adds an element of simplicity to guidelines by combining all “atherogenic lipoproteins” (apoB) or “atherogenic cholesterol” (non-HDLc) into a single marker. There are, however, significant barriers to replace LDLc by non-HDLc or apoB as a primary biomarker for management of dyslipidemia-related risk (135) (Table 6).

What makes a good marker of atherogenic lipoproteins? It should be easily measured in a nonfasting sample with a low cost and accurate procedure, and should not increase healthcare expenses to identify patients in whom CVD prevention strategies or therapies will be cost-effective. For monitoring purpose, the test should not only be able to predict clinically significant events but also respond to changes in the condition or treatment. None of the present tests in question has been completely validated according to key criteria to become a medically useful test, as defined by the EFLM Working Group for Test Evaluation (136). Although the analytical performance of apoB measurements is superior to measurements or calculations of LDLc and even non-HDLc, and the clinical performance of apoB testing may be of added benefit over standard lipid assessment in selected patients, more definitive evidence in rigorously performed outcome studies is needed to confirm that apoB testing will effectively lead to reduced CVD events (clinical effectiveness) and health-economic costs (cost-effectiveness) compared with LDLc-targeted strategies (Table 7). These key test components should be validated in the setting of novel therapies aiming at very low LDLc targets <50 mg/dL (1.3 mmol/L).

A well-defined unmet clinical need should act as the architect for biomarker test development (137). Desirable clinical performance specifications should be pre-defined (for which rate of misclassification and/or mis-

treatment is acceptable) for each test indication because they determine the analytical performance specifications (136). Clinical studies can then be designed to evaluate clinical and cost-effectiveness of the test to improve health and economic outcomes (138). In randomized controlled trials of diagnostic investigations, patients are randomized to receive the new test or the standard test, and the impact on clinical decision for detecting or treating more patients at high risk (i.e., not detected or treated when using the standard test) is assessed (138). Biomarker tests per se do not bear directly on health outcomes. Investigations guide the decisions and actions of clinicians and patients, and it is these that directly impact on health outcomes. New tests can improve outcomes by optimizing the selection of treatment, through more accurate risk classification or prediction of CVD or CVD outcomes (i.e., mortality, morbidity, and complication rates), or by offering other patient benefits (i.e., more appropriate treatment options, the impact of medical care on patient well-being, quality of life) (138). Indeed, the emerging approach of precision medicine (or personalized medicine) requires novel biomarkers for targeted therapies, tailored to the individual patient's condition. Operational outcomes can include time to test results, time to treatment, and other operational advantages such as nonfasting blood sampling (19). Economic outcomes include the cost of the test and all downstream consequences on healthcare, such as the financial benefits of morbidity avoided, quality-adjusted life-years gained, or reductions in length of hospital stay (136, 138).

The use of non-HDLc, apoB, or LDLP would be cost-effective if these tests provide information to guide therapy to reduce the risk (and cost) of CVD events more than standard therapy guided by LDLc alone. In 1 meta-analysis, it was estimated that a non-HDLc targeting strategy would prevent approximately 300 000 more CVD events than an LDLc strategy, whereas an apoB strategy would prevent approximately 500 000 more events than a non-HDLc strategy in the US adult population over a 10-year statin treatment period (94). Recent studies on the cost-effectiveness of LDLP-guided statin therapy, either alone or in combination with LDLc, showed potential cost-saving because of reductions in medical expenses from fewer CVD events, and this approach was estimated to increase quality-adjusted life-years compared with standard LDLc-alone treatment (139–141). LDLP-guided therapy was expected to result in LDLc reductions that exceed those guided by LDLc alone because of the use of higher doses of statins to achieve LDLP goals (139–141). With the availability of inexpensive generic statins, the cost-effectiveness of intensifying pharmacological intervention aiming to reduce non-HDLc, apoB, or LDLP will be enhanced.

Table 6. Strengths, weaknesses, opportunities, and threats (SWOT analysis) of LDLC, non-HDLc, apoB, and LDLP.

LDLC	Non-HDLc	apoB	LDLP
Strengths			
Widely available laboratory assays for dLDLC measurement or cLDLC calculation Clinical performance: strong evidence-based, causal risk factor Clinical effectiveness: LDLC-targeted treatment reduces risk	Not dependent on TG variability Can always be calculated in the nonfasting state Includes remnant cholesterol	Unequivocally defined protein International standard available Analytical performance: fasting and nonfasting test accuracy Fully automated test; can be easily integrated in widely available laboratory autoanalyzer platforms Diagnostic performance for characterization of complex, mixed dyslipidemias	Measures LDL particle number and size Often provides simultaneous quantification of VLDL, IDL, LDL, HDL particle numbers and size, and in some cases other metabolic or inflammatory biomarkers, in 1 single run of the assay
Weaknesses			
dLDLC measurement errors in dyslipidemic samples and samples from diseased patients cLDLC influenced by HDLC measurement errors cLDLC influenced by postprandial TG variability; invalid at TG >400 mg/dL (4.5 mmol/L) cLDLC and dLDLC influenced by increased Lp(a) Manufacturer-dependent nonspecificity bias compared with reference method	HDLC measurement errors in dyslipidemic samples Different assays for HDLC affect between-laboratory measurement variability Arbitrary risk cutpoints and treatment targets, not validated for clinical performance	Controversial clinical performance vs LDLC and non-HDLc for risk estimation in general populations due to high correlations with TC and LDLc Arbitrary risk cutpoints and treatment targets No consistent evidence of significant population health-economic benefit of intensified or novel therapies aiming to reduce apoB	No standardization Inconsistent agreement between different methods Expensive and/or not widely available technology Not easily integrated in high-throughput laboratory operations
Opportunities			
Novel therapies appear to safely reduce LDLC to very low concentrations (long-term follow-up is limited) Novel therapies confirm the LDL hypothesis "the lower the better" Increasing awareness and demand to screen for familial hypercholesterolemia Improved cLDLC equations published	Healthcare budget: no additional cost Increasing use of nonfasting blood samples Additional risk reclassification by discordance analysis vs LDLc Clinical utility particularly when LDLc is low or TGs are increased (needs to be validated)	Increasing prevalence of obesity, diabetes, and atherogenic dyslipidemias with increased apoB Additional risk reclassification by discordance analysis vs LDLc (and in some studies vs non-HDLc) Clinical utility particularly when LDLc is low or TGs are increased (needs to be validated) Emerging mass spectrometry applications in the clinical laboratories, enabling more precise and multiplex apolipoprotein tests	Increasing prevalence of atherogenic dyslipidemias with increased LDLP and VLDLP Additional risk reclassification by discordance analysis vs LDLc Emerging evidence of cost-effectiveness by metaanalyses Automated NMR assays are being developed
Threats			
Residual on-treatment CVD risk, not explained by LDLc Increasing prevalence of obesity, diabetes, and atherogenic dyslipidemia, in which LDLc is less predictive Uncertain analytical performance at very low concentrations obtained with novel therapies	No conceptual understanding by most practicing physicians and patients	Potential increases in estimated healthcare costs Poor goal attainment rates on statin therapies, including high-dose statins No conceptual understanding by most practicing physicians and patients	Assays not widely available in clinical laboratories No conceptual understanding by most practicing physicians and patients

Consensus-based recommendation. The ideal biomarker(s) of atherogenic lipoproteins should be validated for analytical performance, clinical performance, clinical effectiveness, and cost-effectiveness to justify its/their use in clinical practice in different well-defined settings: screening, dyslipidemia diagnosis, treatment choice, and follow-up. For now and until these issues are clarified, non-HDLc is the best choice, as it incurs no additional expense to the patient or health system. A cost-efficient approach is to measure 3 markers (TC, TG, HDLC) and to calculate cLDLC and non-HDLc. Clinical laboratories should proactively calcu-

late and report non-HDLc together with cLDLC on all lipid profiles (CBR5) (142).

Conclusion

The principal aim of laboratory medicine is to provide a high-quality service that delivers unequivocal test information and improves patient outcomes across the continuum of care. Laboratory medicine is a key component of models of care for all types of lipid disorders (142). We have provided CBRs for improving the use of the lipid profile to assess CVD risk conferred by atherogenic lipo-

Table 7. Contemporary evidence for the medical use of LDLC, non-HDLc, apoB, and LDLP as biomarkers based on essential criteria.

Test characteristics	LDLC	Non-HDLc	ApoB	LDLP
Analytical performance^a				
Precise assays	Yes	Yes	Yes	Yes
Accurate assays (method independency)	No	No	Yes	No
Nonfasting measurement possible	With TG <4.5 mmol/L	Yes	Yes	Yes
Widely accessible assays	Yes	Yes	Yes	No
High throughput and rapid turnaround	Yes	Yes	Yes	Yes
Reasonable operational costs	Yes	No extra cost	Yes	Not yet
Clinical performance^b				
Robust associations with incident CVD?	Yes	Yes	Yes	Yes
Novel information beyond existing markers?	(Reference)	Yes	Yes	Yes
Validated decision limits?	No	No	No	No
Clinical effectiveness^c				
Superiority to existing tests?	(Reference)	Probably	Probably	Probably
Modifiable risk association (treatment target)?	Yes	Yes	Yes	Yes
Biomarker-guided treatment reduces CVD risk?	Yes	Probably	Probably	Unknown
Cost-effectiveness^d				
Biomarker-guided treatment saves healthcare costs?	Yes	Unknown	Unknown	Unknown

^a Analytical validity: ability of the test to conform to predefined quality specifications to measure the marker of interest.
^b Diagnostic or prognostic accuracy: ability of the test to consistently detect patients with a high risk for developing CVD.
^c Clinical utility: ability of the test to improve health outcomes of the patient under standard clinical care.
^d Health-economic advantage of introducing the test in medical practice (value for money).

proteins (Table 2). EQA programs, which structurally evaluate laboratory test performance, demonstrate that improvements are necessary (47, 48). New therapies demand accuracy of dyslipidemia testing at very low LDLC concentration ranges. Incorrect diagnosis and mismanagement of treatment, which are based on laboratory measures, are both costly to society and harmful to the patient. Even modest improvements in laboratory testing to predict risk of a disease as common as CVD translate into thousands of people who may be treated more appropriately and could benefit. Therefore, it is essential that diagnostic test results produced in laboratories worldwide are comparable across different medical facilities, enabling unequivocal diagnosis, treatment, and monitoring of patients. This prerequisite is increasingly recognized by European Conformity (CE) marking and Food and Drug Administration regulatory bodies in their patient-focused risk assessment during premarket approval of in vitro diagnostic medical devices (136). To that end, global standardization and harmonization of lipid tests should be the key for sustainable patient care with universal application of desirable values and decision cutpoints, as well as preparing for future exchange and interoperability of electronic health records (142, 143).

Some questions remain unanswered and need further investigation (Table 8). In hypertriglyceridemic patients, several analytical and clinical performance limitations make LDLC an unreliable marker of atherogenic dyslipidemia; hence, there is a possibility of undertreatment. ApoB has the potential to be standardized across laboratories, which is not possible for LDLC and non-HDLc with the current CDC standardization program. Non-HDLc and apoB have the potential to address clinical needs unmet with LDLC testing, and they can always be used in nonfasting samples. To combine the strengths and to compensate for the weaknesses of the different markers, currently it seems the best strategy to consider is the use of LDLC, non-HDLc, and apoB as complementary rather than competitive markers of CVD risk and therapeutic response. Nonfasting non-HDLc can be used to assess the incremental risk of remnant lipoproteins, and apoB can detect increased LDLP often missed with LDLC alone. This suggestion is consistent with several guidelines and consensus documents that propose to use non-HDLc or apoB as a secondary treatment target in the treatment of high-risk or very high-risk patients with mild to moderate hypertriglyceridemia, defined as 2 to 10 mmol/L (175–880 mg/dL) (CBR8) (14). If the primary target LDLC is at goal but non-HDLc or apoB

Table 8 Unanswered questions and recommendations for future research.

Unanswered questions (Q)	
Q1	Which (measured or calculated) LDLC method provides the best proxy for the gold standard measurement (ultracentrifugation) to ensure adequate translation of LDLC test results to the atherogenic risk of “true” LDL particles?
Q2	Are guideline-recommended LDLC and non-HDLc goals used in clinical practice (based on Friedewald-cLDLC and non-HDLc calculations in study populations using the older HDLC precipitation methods) still applicable to LDLC and non-HDLc values as currently reported (i.e., as measured or calculated using contemporary assays)?
Q3	Can follow-up of lipid-lowering therapies be simplified using a single-marker, nonfasting apoB assay to replace the traditional lipid profile to obtain at least equal, or even better, assessment of dyslipidemia-related risk?
Q4	Can multimarker test panels of apolipoproteins and/or lipoprotein particles and subclasses provide precision medicine-individualized assessment of dyslipidemia-related risk in the patient?
Future research (FR) action points to address the above questions	
FR1	Assay kit manufacturers and standardization programs should be closely linked to optimize the assays, or select only 1 best possible chemical procedure and type of assay, to ensure accurate and unequivocal measurements of LDLC and HDLC, including low concentrations, in the fasting and nonfasting state in both normotriglyceridemic and hypertriglyceridemic blood samples (Q1)
FR2	New generation assays should be validated for clinical performance to discriminate between high- and low-risk patients, e.g., by reporting risk reclassification in observational studies using the net reclassification index (Q2)
FR3	Clinical and cost-effectiveness studies should be designed to determine the health outcomes and economic implications of apoB-based treatment of high-risk patients (or subgroups of patients), relative to the standard care of LDLC management (Q3)
FR4	NMR-based lipoprotein particle/subclass numbers and size measurement procedures should be standardized to ensure direct comparability of NMR data for the development of universally applicable guidelines and decision cutpoints (Q4)
FR5	Multiplex LC-MS/MS apolipoprotein profiles (including apoA-I, B, C-I, C-II, C-III, and E) rather than single apoB tests should be further developed, standardized, and evaluated for the creation of a personalized clinical pathway: clarification of unmet clinical need(s) and diagnostic accuracy to identify the dyslipidemias of interest (Q4)

is still high, attainment of all 3 targets will require intensified lipid-lowering therapy, lifestyle (re)inforcement, and/or additional TG-lowering drugs (e.g., fibrate or omega-3 fatty acids) (1).

Which of the biomarkers to choose as secondary target: non-HDLc or apoB? To help train clinicians and patients in gaining an understanding of the concept and advantage of apoB (or LDLP) testing beyond LDLc, we need to gently transition into this process. To that end, as a first step we should move to include non-HDLc into the report of every lipid profile. Diabetes and abdominal obesity, the disorders that underlie the clinical expression of polygenic hypertriglyceridemia, are attaining epidemic proportions (14); hence, apoB and LDLP tests will likely become more and more useful in the future. This underscores the need to standardize and harmonize innovative biomarkers such as NMR-based lipoprotein particles or LC-MS/MS proteomics, which have the potential to become innovative medical tests. To be prepared for the future of personalized medicine, obviously the

most important challenge of apoB is to validate its use as part of a multiplex MS/MS-based apolipoprotein profile rather than a single marker test. These technologies provide complementary clinical information regarding the complex molecular basis of polygenic hypertriglyceridemias and, as such, will contribute by identifying better and individualized treatment options for dyslipidemic patients.

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critically reviewed the literature while the second meeting reviewed additional literature and scrutinized the first draft of the joint consensus statement. The recommendations should be considered as consensus-based because evidence was not always available or inconsistent. All Panel members agreed to conception and design, contributed to interpretation of available data, all suggested revisions for this document, and all members approved the final document before submission.

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